BF-1522
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RESEARCH ARTICLE Indian Journal of Animal Research

The Effect of the Addition of Co-Q10 on the Preservation of Boar Semen at 17°C

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ABSTRACT

Background: With the increasing application of artificial insemination technology in boars, breeding efficiency is largely determined by the quality of semen preservation. With the prolonged storage of semen at room temperature *in vitro*, excessive reactive oxygen species (ROS) damage organelles and plasma membranes and ultimately lead to a decline in semen quality.

Methods: In order to improve the preservation of semen at 17°C, this experiment investigated the effect of different concentrations (0, 10, 15, 25, 35 μg/mL) of coenzyme Q10 (Co-Q10) in Modena dilution on the quality of boar semen during storage at 17°C. The sperm motility, mitochondrial activity, total antioxidant (T-AOC), superoxide dismutase (SOD) and malondialdehyde (MDA) were assessed at 17°C at different times (0, 1, 2, 3, 4, 5 d).

Result: The results showed that the addition of Co-Q10 has a positive effect during the preservation of boar semen at 17°C. On the 5th day, the sperm motility of the 25 μg/mL Co-Q10 group was 73.80% and the mitochondrial activity was 82.94%, which was significantly higher (P<0.05) than that of other groups. In terms of antioxidant levels, the MDA content of the 25 μg/ml and 35 μg/ml Co-Q10 groups on the 5th day was significantly lower (P<0.05) than that of the other groups. At the same time, the 25 μg/ml group could effectively enhance the activity of antioxidant enzymes and the SOD and T-AOC were 17.33 U/mL and 3.17 U/mL on the 5th day significantly higher than the other groups (P<0.05). The results showed that the most appropriate concentration of Co-Q10 when storing boar semen at 17°C was 25 μg/ml.

Key words: Antioxidant, Boar semen, Co-Q10.

INTRODUCTION

Artificial insemination technology has been widely used in boar production and breeding; it has contributed significantly to breed improvement and selection (Knox,2016). Semen quality is one of the key factors that determine the process of artificial insemination. ROS accumulate over time during the preservation, causing a large amount of ROS that cannot be neutralized to undergo lipid peroxidation reactions with phospholipids on cell membranes, destroying their structures (Allai, 2018). Boar sperms are more sensitive and susceptible to oxidative damage than other species (Dong *et al* . 2021). Therefore, adding effective antioxidants during the preservation process is the most effective and convenient method to reduce the degree of damage to boar sperm.

Co-Q10 is a lipid-soluble quinone compound that is an antioxidant with strong biological activity (Alahmar *et al* .2021) . When this lipophilic antioxidant is added to the semen, it can spread directly to the polyunsaturated lipid chain present in the plasma membrane to generate energy and prevent ROS from affecting the sperm's structure and function (Appiah *et al.* 2020). At present, Co-Q10 has been proved to have an antioxidant protective effect on sperm in humans (Alahmar *et al.* 2021), dogs (Kobayashi *et al.* 2021), broiler (Sharideh *et al.* 2020) and other experiments. It was also confirmed that CoQ-10 could effectively inhibit lipid peroxidation in rooster semen (Masoudi *et al.* 2019) supplementation experiments that are also sensitive to ROS.

The quality of spermatozoa is seriously affected due to the inevitable oxidative damage during storage at 17°C. Co-Q10 can effectively reduce the damage during semen preservation as a strong and effective antioxidant. In the present study, different concentrations of Co-Q10 were added to study the effect of Co-Q10 on semen. A control group and 4 concentration groups were set up in this experiment. The sperm motility, mitochondrial activity, T-AOC, SOD activity and MDA content were tested and analyzed to explore the effect and appropriate concentration of boar semen.

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How to cite this article: Li, J.C., Zhang, Q., Liu, Z.G., Wang, Q., Li, Y.B. and Wei, G.S. (2022). The Effect of the Addition of Co-Q10 on the Preservation of Boar Semen at 17°C. Indian Journal of Animal Research. DOI: 10.18805/IJAR.BF-1522.

Submitted: 23-03-2022 **Accepted:** 10-06-2022 **Online:** 25-06-2022

MATERIALS AND METHODS

The indicators of sperm motility, mitochondrial activity, T-AOC, SOD and MDA were measured at 0, 1, 3 and 5 days

of semen preservation. The sperm motility can directly reflect sperm quality and reproductive status of the boar and determine the fertilization ability. ROS generated by sperm metabolism can cause lipid peroxidation of sperm membranes and T-AOC, SOD and MDA are antioxidant indicators, which can reflect the antioxidant capacity of semen The experimental records were taken and each experimental group was repeated 4 or 5 times.

Except otherwise stated, all reagents used for this experiment were purchased from Sigma. The boar semen was collected from 6 boars on the porcine farm of Heilongjiang Bayi Agricultural University.

Preparation of spermatozoa

The boar semen used in the experiment was collected from 6 healthy Landrace boars aged 12-16 months in the experimental pig farm of Bayi Agricultural Reclamation University in Heilongjiang. The fresh semen retrieved from the experimental boar farm was centrifuged at 1800 RPM for 5 min in a centrifuge. The supernatant was removed, diluted and mixed in proportion and a haemocytometer detected the sperm concentration in the semen. Then, the concentration of the original semen was adjusted to 1×10^8/mL with Modena dilution and then divided into 10 mL centrifuge tubes with different concentrations of Co-Q10, placed in a 17°C incubator mixed every 12 hours.

Determination index

Sperm motility was detected by a MAILANG automatic sperm analyzer every 24 h during semen preservation. The diluted semen was shaken before detection with 15 µL pipetted from the dilution mix and preheated in a 37°C carbon dioxide incubator for 15 min. This was dropped on a glass slide and covered with a coverslip. After testing, records of the experiment were taken.

Semen (500 µL) was taken, washed twice with PBS, resuspended and the concentration was adjusted to 1×10^6/mL. We added 5 µL of JC-1 staining working solution to the suspension and incubated it in a 37°C water bath for 30 min in the dark. Then, the suspension was washed once with PBS, resuspended and observed under a fluorescence microscope. The tail of sperm with high mitochondrial activity was orange-red.

The determination of T-AOC, SOD and MDA of sperm was carried out according to the instruction of the kits.

Statistical analysis

Data from four replicated trials were analyzed by one-way ANOVA using the STATVIEW 5.0 software (Abacus Concepts, Inc., Berkeley, CA, USA). If the *P*-value was smaller than 0.05 in the ANOVA, Bonferroni/Dunn test was carried out using the same program. All data were expressed as means± SD. Findings were considered significantly different at $P<0.05$.

RESULTS AND DISCUSSION

The effects of adding different concentrations of Co-Q10 to diluted semen on sperm motility are shown in Table 1. It can be seen from Table 1 that the motility of boar semen decreased with the increase in storage time. On the 1st day of storage, the viability of the 4 groups supplemented with Co-Q10 was significantly ($P>0.05$) better than the control group, but there was no significant difference between the 10 µg/mL, 15 µg/mL and 25 µg/mL groups. On the 3rd to 5th day of storage, the addition amount of 25 µg/mL Co-Q10 group was significantly ($P<0.05$) higher than the other experimental groups. The effects of adding different concentrations of Co-Q10 to the semen dilution on sperm mitochondrial activity are shown in Table 2. On the 1st day, the percentage of spermatozoa with mitochondrial activity

Table 1: Effect of adding Co-Q10 on sperm motility in basal dilution (%).

Group (µg/mL)	Sperm motility (%)			
	0 d	1 d	3 d	5 d
0	89.90±0.43^a	79.9±0.35^c	73.60±0.89^c	61.10±0.70^d
10	89.90±0.43^a	83.4±0.19^{ab}	75.80±1.41^b	67.70±1.06^{bc}
15	89.90±0.43^a	84.05±0.35^{ab}	76.30±0.89^b	68.30±0.56^b
25	89.90±0.43^a	84.7±0.56^a	79.25±0.49^a	73.80±0.70^a
35	89.90±0.43^a	82.7±1.41^b	74.85±0.77^{bc}	66.35±0.77^c

In the columns, different letters represent a significant difference ($P<0.05$) between groups.

Table 2: Effect of adding Co-Q10 on mitochondrial activity in basal dilution (%).

Group (µg/mL)	Mitochondrial activity(%)			
	0 d	1 d	3 d	5 d
0	96.31±0.72^a	93.82±1.12^b	87.80±0.87^c	73.36±1.23^d
10	96.31±0.72^a	94.14±0.78^a	90.42±0.54^b	81.53±0.65^{bc}
15	96.31±0.72^a	94.62±0.93^a	89.63±0.76^b	82.22±0.63^b
25	96.31±0.72^a	94.76±0.54^a	92.44±0.43^a	84.65±0.42^a
35	96.31±0.72^a	94.00±1.03^a	90.76±0.78^b	80.26±0.28^c

In the columns, different letters represent a significant difference ($P<0.05$) between groups.

in each experimental group was significantly (P<0.05) higher than that in the control group (Table 2). On the 3rd day, the percentage of spermatozoa with mitochondrial activity in the 25 μg/mL Co-Q10 group was significantly (P<0.05) higher than the other groups but all of the experimental groups were significantly (P<0.05) higher than the control group. When the semen was stored on the 5th day, the percentage of spermatozoa with mitochondrial activity was still significantly (P<0.05) higher in the 25 μg/mL Co-Q10 group than in the other groups but the control group was the lowest.

Although the frequency of sperm motility can be slowed down during storage at 17°C, it will still be metabolized and consumed during the storage process (Torres *et al* . 2018). During the metabolic process, the excessive accumulation of ROS produced by the sperm tail and the ROS produced by the plasma membrane can trigger sperm oxidative stress (Aitken, 2017; Vongpralub *et al* . 2016). This experimental study found that adding Co-Q10 can improve sperm motility and mitochondrial activity and that it can be stored at 17°C for at least 120 h. Similar results were reported by Kobayashi Masanori (2021) in the dog sperm treatment experiment. Lin and Liu (2021) reported that sperm motility was significantly improved in the group with Co-Q10 supplementation. Masoudi (2018) also reported that supplementation with Co-Q10 resulted in greater sperm motility and mitochondrial activity. Sperm motility is a key factor in determining fertilization and plays a key role in whether sperm can combine with an egg to fertilize it. The spermatozoa were more easily oxidized after being stored at 17°C for 72 h than the sperm motility of the group treated with 25 μg/mL. Co-Q10 in the dilution was significantly higher than the other experimental and control groups on the 3rd and 4th days of storage, indicating that this concentration is an effective dose for enhancing motility. Studies have shown that sperm motility is positively correlated with mitochondrial activity (Malo *et al.* 2012). The results of this experiment showed that the mitochondrial activity of each experimental group was significantly higher than that of the control group during the five days and the percentage of sperm with high mitochondrial activity in the 25 μg/mL Co-Q10 treatment group was the highest throughout the storage period.

The effects of different concentrations of Co-Q10 in semen dilutions on T-AOC activity in semen are shown in Fig 1. On the first day of storage, the T-AOC activity of semen increased with the increase of Co-Q10 concentration and the test group was significantly(P < 0.05) higher than the control group (Fig 1). On the 3rd and 5th day of storage, the T-AOC activity of all the experimental groups was significantly(P < 0.05) higher than the control group and the 25 μg/mL Co-Q10 group had the strongest T-AOC activity and was significantly higher than 10 μg/mL, 15 μg/mL and 30 μg/mL Co-Q10 group.

This experiment proved that on the 5th day of storage, the T-AOC in the semen when the concentration of Co-Q10 used was 25 μg/mL were significantly (P<0.05) higher than those of other experimental groups and control groups. Wu *et al.* (2020) proved this with similar results and the T-AOC was improved markedly in supplemented groups with Co-Q10. Moreover, the sperm motility and mitochondrial activity of the 25 μg/mL Co-Q10 group on the 5th day of storage were also excellent, which could be related to be the effect of the antioxidant capacity of Co-Q10.

The effects of different concentrations of Co-Q10 on the SOD activity in semen are shown in Fig 2. In the 1st day, the SOD activity of the 25 μg/mL Co-Q10 group was significantly higher (P<0.05) than the other groups (Fig 2). On the third day, the SOD activity of the 25 μg/mL Co-Q10 group was significantly higher than that of the other groups and the other experimental groups were also significantly higher than the control group. On the 5th day of storage, the SOD activity of the 25 μg/mL Co-Q10 group was also significantly higher (P<0.05) than the other groups. Overall, adding 25 μg/mL Co-Q10 group could improve the SOD activity in semen.

SOD can highly specifically remove superoxide anion groups in semen and plays an important antioxidant role in

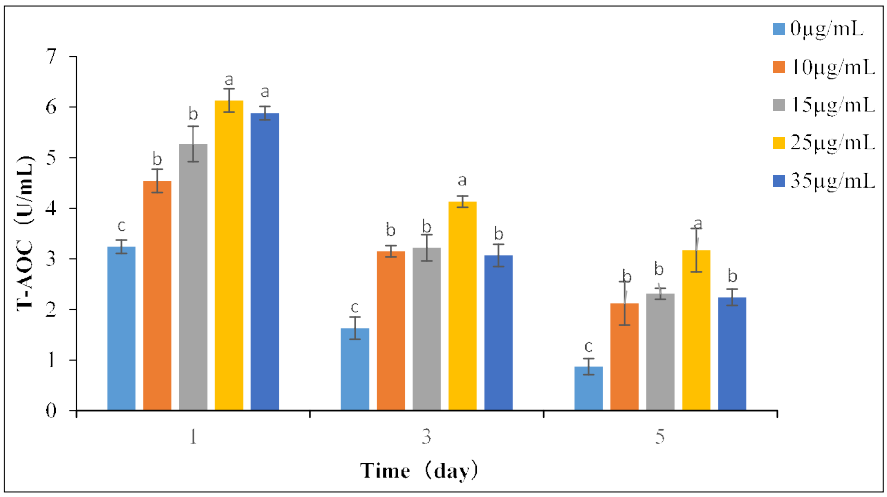

Fig 1: Effect of adding Co-Q10 on T-AOC in basal dilution.

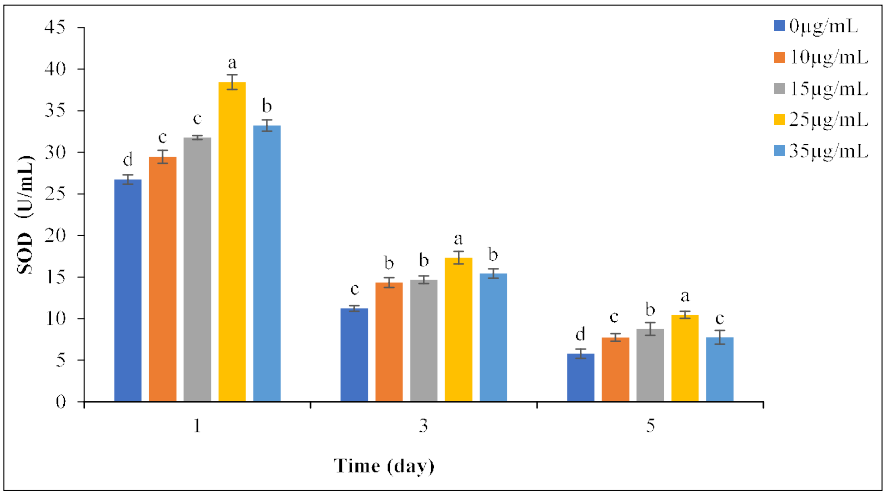

Fig 2: Effect of adding Co-Q10 on SOD activity in basal dilution.

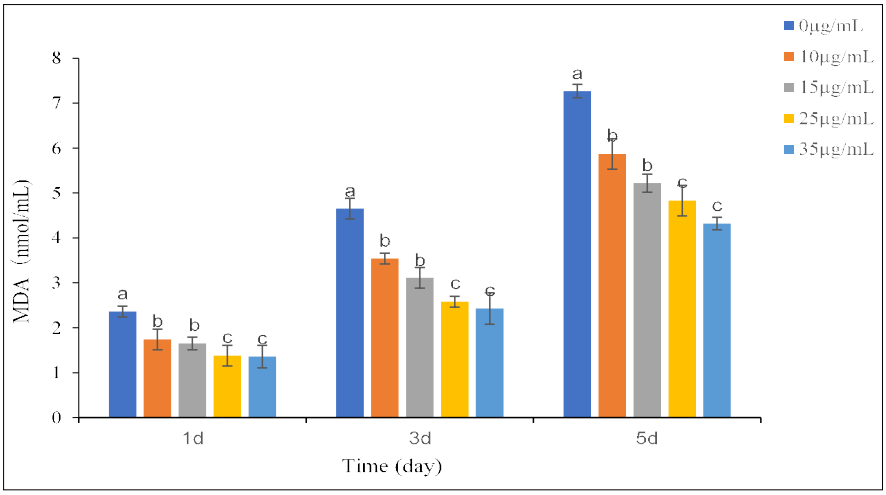

Fig 3: Effect of adding Co-Q10 on MDA content in basal dilution.

organisms (Torres *et al.* 2021). Fouad *et al.* (2011) revealed that Co-Q10 could suppress oxidative stress in the testis by inhibiting lipid peroxidation and enhancing antioxidant enzyme activity. Therefore, reducing SOD activity will make scavenging superoxide anion free radicals worse and the lipid peroxidation products caused will destroy the proteins and enzymes of sperm cells and reduce sperm quality.

The effects of different concentrations of Co-Q10 on the MDA content in semen are shown in Fig 3. On the 1st, 3rd and 5th days of storage, the content of MDA in the experimental group added with Co-Q10 was significantly lower (P<0.05) than the control group. And the higher the concentration of Co-Q10, the lower the MDA content and the groups with 25 μg/mL and 35 μg/mL had the best effect.

During the preservation of semen *in vitro*, due to the limited antioxidant capacity of the antioxidative enzyme system of the semen itself (Anzar *et al.* 2011), oxidative stress is one of the main reasons for the decrease in the success rate of artificial insemination. An important lipid peroxidation product is MDA and its content will directly affect the quality of semen preservation (Aitken, 2017). The results of this experiment showed that with the increase of the concentration of Co-Q10, the MDA content in semen showed a downward trend and the groups with 25 μg/mL and 35 μg/mL Co-Q10 had the best effect. In addition, as a marker of lipid peroxidation, the MDA levels were significantly reduced after Co-Q10 treatment, as published by Bakhshayeshkaram (2018). This indicates that the addition of Co-Q10 reduces sperm lipid peroxidation and protects sperm.

CONCLUSION

Adding Co-Q10 to boar semen dilution can effectively prolong the storage time of boar semen at 17°C. On the 5th day, the sperm motility and mitochondrial activity of the 25 μg/mL group were significantly higher than the other groups, which was the most favorable for semen preservation. Meanwhile, adding the Co-Q10 can also improve the antioxidant level and preservation quality of boar semen. On the 5th day, the SOD and T-AOC ability were the strongest in the 25 μg/mL group. In terms of MDA content, the 25 μg/mL and 35 μg/mL Co-Q10 groups were significantly lower than the control group on the 5th day of storage. Therefore,

the appropriate concentration of Co-Q10 in boar semen stored at 17°C is 25 μg/mL.

ACKNOWLEDGEMENT

This project was supported by the Postdoctoral scientific research developmental fund of Heilongjiang Province (No.LBH-Q18100); The Doctoral Starting up Foundation of Heilongjiang Bayi Agricultural University (No.XYB-2016-04;No.XYB201910); Heilongjiang Bayi Agricultural University Support Program for San Zong (No. ZRCPY202107). We would like to thank Dr Samson O. ADENIRAN (Department of Biological Sciences, College of Basic and Applied Sciences, Mountain Top University Ibafo, Ogun State Nigeria) for English language editing.

Conflict of interest: None.

REFERENCES

Aitken, J.R. (2017). Reactive oxygen species as mediators of sperm capacitation and pathological damage. Molecular Reproduction and Development . 84(10): 1039-1052.

Alahmar, A.T., Calogero, A.E., Singh, R., Cannarella, R., Sengupta, P., Dutta, S.(2021). Coenzyme Q10, oxidative stress and male infertility: A review. Clin Exp Reprod Med. 48(2): 97-104.

Alahmar, A.T., Sengupta, P. (2021). Impact of coenzyme Q10 and selenium on seminal fluid parameters and antioxidant status in men with idiopathic infertility. Biol. Trace Elem. Res. 199(4): 1246-1252.

Allai, L. (2018). Supplementation of ram semen extender to improve seminal quality and fertility rate. Animal Reproduction Science. 192: 6-17.

Anzar, M., Kroetsch, T., Boswall, L. (2011). Cryopreservation of bull semen shipped overnight and its effect on post-thaw sperm motility, plasma membrane integrity, mitochondrial membrane potential and normal acrosomes[J]. Animal Reproduction Science. 126(1-2):23-31.

Appiah, M.O., Asante-Badu, B., Zhao, J. (2020). Possible protective mechanisms of coenzyme Q10 action on spermatozoa during cryopreservation or cooled-stored condition. Co Letters. 41(5): 246-256.

Bakhshayeshkaram, M., Lankarani, K.B., Mirhosseini, N., Tabrizi, R., Akbari, M., Dabbaghmanesh, M.H., Asem, Z. (2018). The effects of coenzyme Q10 supplementation on metabolic profiles of patients with chronic kidney disease: A systematic review and meta-analysis of randomized controlled trials. Curr. Pharm. 24: 3710-3723.

Dong, L., Zhang, W.Y., Tian, X.K., Xiao, Z.T., Zhao, X., Lin, F., Du, R.L., Gong, S.Y., Yu, Y.T. (2021). Hydroxytyrosol effectively improves the quality of pig sperm at 17°C. Theriogenology. 177: 172-182.

Fouad, A.A., Al-Sultan, A., Yacoubi, M.T. (2011). Coenzyme Q10 counteracts testicular injury induced by sodium arsenite in rats. Eur. J. Pharmacol. 25;655(1-3): 91-8.

Knox, R.V. (2016). Artificial insemination in pigs today. Theriogenology. 85(1): 83-93.

Kobayashi, M., Tsuzuki, C., Kobayashi, M. (2021). Effect of supplementation with the reduced form of coenzyme Q10 on semen quality and antioxidant status in dogs with poor semen quality: Three case studies. Veterinary Medicine and Science. 83(7): 1044-1049.

Lin, Y.S., Liu, C.Y., Chen, P.W., Wang, C.Y., Chen, H.C. and Tsao, C.W. (2021). Coenzyme Q10 amends testicular function and spermatogenesis in male mice exposed to cigarette smoke by modulating oxidative stress and inflammation. American Journal of Translational Research. 13(9): 10142-10154.

Malo, C., Gil, L., Cano, R., Martínez, F., García, A. and Jerez, R.A. (2012). Dimethylformamide is not better than glycerol for cryopreservation of boar semen. Andrologia. 44(Supplement s1): 605-610.

Masoudi, R., Sharafi, M., Pourazadi, L. (2019). Improvement of rooster semen quality using coenzyme Q10 during cooling storage in the Lake extender. Cryobiology. 88: 87-91.

Masoudi, R., Sharafi, M., Zare Shahneh., A, Kohram, H., Nejati, E., Karimi, H., Khodaei, M., Shahverdi, A. (2018). Supplementation of extender with coenzyme Q10 improves the function and fertility potential of rooster spermatozoa after cryopreservation. Anim Reprod Sci. 198:193-201.

Sharideh, H., Zeinoaldini, S., Zhandi, M. (2020). Use of supplemental dietary coenzyme Q10 to improve testicular function and fertilization capacity in aged broiler breeder roosters. Theriogenology. 142: 355-362.

Torres, M. A., Monteiro, M. S., Passarelli, M. S., Papa, F. O. andrade, A. (2018). The ideal holding time for boar semen is 24 h at 17°C prior to short-cryopreservation protocols. Cryobiology. 86: 58-64.

Torres, M. A., Rigo, V., Leal, D. F., Pavaneli, A., Andrade, A. (2021). The use of resveratrol decreases liquid-extend boar semen fertility, even in concentrations that do not alter semen quality. Research in Veterinary Science. 136: 360-368.

Vongpralub, T., Thananurak, P., Sittikasamkit, C., Chuawongboon, P., Duangjinda, M., Boonkum, W. (2016). Comparison of effects of different antioxidants supplemented to long-term extender on boar semen quality following storage at 17°C . Thai Journal of Veterinary Medicine. 46(1): 119-126.

Wu, X., Liang, S., Zhu, X., Wu, X. and Dong, Z. (2020). CoQ10 suppression of oxidative stress and cell senescence increases bone mass in orchiectomized mice. American Journal of Translational Research. 12(8): 4314-4325.