Taiwan Veterinary Journal, Vol. 44, No. 3 (2018) 111–117
DOI: 10.1142/S1682648518500051

EFFECTS OF AUTOLOGOUS SERUM EYE DROPS FOR TREATMENT OF KERATOCONJUNCTIVITIS SICCA IN DOGS

Han-Syuan Lin*, Shiun-Long Lin*,† and Feng-Jen Chang†,‡
*Department of Veterinary Medicine
College of Veterinary Medicine
National Chung Hsing University
No. 250-1, Guoguang Rd.
Taichung City 40227, Taiwan
†Veterinary Medical Teaching Hospital
National Chung Hsing University
No. 250-1, Guoguang Rd.
Taichung City 40227, Taiwan

Received 14 June 2018
Accepted 3 July 2018
Published 10 August 2018

ABSTRACT

In humans, autologous serum (AS) eye drops has been applied for the treatment of refractory keratoconjunctivitis sicca (KCS) for several decades. However, there are few researches to investigate the AS eye drops in dogs with KCS. The objective of this study was to evaluate the effects of AS eye drops on treatment of KCS in dogs. Eighteen eyes of ten client-owned dogs with refractory KCS were used in this study. Schirmer tear test (STT), tear film breakup time (TBUT), fluorescein (FL) staining score, and Rose Bengal (RB) staining score were used to measure the status of cornea prospectively at baseline and 1–3 months after treatment. Additionally, the results were further stratified by their STT value, sex, and age. The results indicated that the mean TBUT, FL staining score, and RB staining score were significantly improved after treatment except STT. In 18 eyes, 77.8% eyes had decreased mucopurulent ocular discharge and 38.9% eyes got wet. Besides, both TBUT and RB staining score were significantly improved in a subgroup of dogs with age less than 9 years old. As far as we know, this study is the first trial to determine the efficacy and safety of 20% AS eye drops for cKCS. In conclusion, AS eye drops seemed to be effective and safe for dogs with KCS, and it could improve tear film stability, ocular surface health, and subjective clinical symptoms, especially in dogs younger than 9 years old.

Keywords: Autologous serum; Canine; Keratoconjunctivitis sicca; Schirmer tear test; Tear breakup time.

‡Corresponding author: Feng-Jen Chang, No. 250-1, Guoguang Rd., Taichung City 40227, Taiwan. Tel: +886 4 2284 0405; Fax: +886 4 2285 2016. E-mail: vacation760913@email.nchu.edu.tw

INTRODUCTION

Canine keratoconjunctivitis sicca (cKCS), also called Canine dry eye syndrome (cDES), is a deficiency of the aqueous component of the tear film resulting in inflammatory condition of the ocular surface.[1,2] Presenting signs with cKCS include blepharospasm, mucopurulent ocular discharge, conjunctivitis, keratitis, and visual disturbances.[1,3] The incidence of cKCS was approximately 1% of the population of dogs presented to North American Veterinary Medical Colleges.[4] Although there were a variety of pathogens for KCS, immune-mediated causes were considered as the most common etiology in clinical practice.[1,5] Artificial tears, topical corticosteroids, topical antibiotics, topical tacrolimus, and topical cyclosporine A were mentioned in conventional treatments of cKCS.[1] Cyclosporine A, the major medicine for refractory cKCS, could inhibit T cell activation and increase tear production.[6] Unfortunately, cyclosporine A was ineffective in about 25% of dogs with KCS.[7–9]

In humans, autologous serum (AS) has been applied to treat refractory KCS for several decades, which comprises some similar components in natural tears that adjust corneal and conjunctival epithelial cell proliferation, differentiation, and maturation.[10–12] This application was first reported in 1984 by Fox *et al.*[13] Thereafter, Tsubota *et al.* demonstrated that pH, osmolality and biomechanical properties of AS eye drops were similar to those of normal tears.[14] The essential ocular surface epitheliotropic factors such as vitamins, growth factors, fibronectin and bacteriostatic components were also found in serum.[14,15] Therefore, AS eye drop could be regarded as a great tear substitute. The other indications for use of AS include persistent epithelial defects, recurrent corneal erosion, neurotrophic keratitis and so on.[16–19]

Up to now, AS eye drops were more often utilized in dogs with corneal epithelial defects. However, there were few researches to investigate the application of AS eye drops in cKCS. As far as we know, this study is the first trial to determine the efficacy and safety of 20% AS eye drops for cKCS. The aim of this study was to evaluate the efficacy and safety of AS eye drops in dogs with KCS.

MATERIALS AND METHODS

This study protocol was reviewed and approved by the Institutional Animal Care and Use Committee at National Chung Hsing University of veterinary medicine (IACUC No. 106-019). This was a prospective study performed by the Ophthalmology Department at the Veterinary Medical Teaching Hospital, National Chung Hsing University between October 2016 and March 2017. Inclusion criteria were diagnosed with cKCS and have been already treated with conventional dry eye treatment for more than 3 months without improvement. The diagnosis of cKCS was evidence of decreased tear production. Moreover, the clinical signs of cKCS included mucopurulent ocular discharge that was frequently adherent to the corneal surface and recurrent conjunctivitis. Dogs with any other ocular inflammation which was not associated with dry eye were excluded from this study, including ocular allergy, eyelid abnormality, uveitis and glaucoma. Medical records consisted of breed, age, sex, body weight, changes in subjective symptoms, and the status of AS eye drops use. Informed consent form was signed by the owner after all information regarding the procedure was provided prior to enrollment of the dog in the study. All study-related drugs and materials were provided to owners without charge. Eighteen eyes of 10 dogs which showed no response to conventional dry eye treatment more than 3 months were enrolled in this study.

In the first clinic visit, complete ophthalmic examinations were performed on the patients. Besides, Schirmer tear test I (STT), tear film breakup time (TBUT), fluorescein (FL) staining score, and Rose Bengal (RB) staining score were accomplished in every subsequent visit. The STT was first carried out by placing a standard filter paper strip into the ventral conjunctival sac one-thirds along from the lateral canthus for 1 min. The TBUT was demonstrated by rinsing fluorescein impregnated strip with sterile normal saline and touching on the dorsal bulbar conjunctiva gently. This test was repeated for at least three times to count the fluorescent tear film breakup time and calculate the mean value. The corneal and conjunctival epithelial were stained using FL dye and RB dye. The staining scores in each region were as follows: 0 = no staining, 1 = staining in partial area, 2 = staining in more than half area, 3 = staining in the entire area. Fluorescein staining score was calculated for the superior cornea, mid-cornea, and inferior cornea and graded from 0 to 9: 0 = intact corneal epithelium without ulceration or erosion, 9 = complete corneal epithelial defect. RB staining score was determined as the sum of those in superior cornea, mid-cornea, inferior cornea, nasal conjunctiva, and temporal conjunctiva and ranged from 0 to 15: 0 = intact corneal and conjunctival epithelium without necrosis or degeneration, 15 = complete corneal and conjunctival epithelial defect. All examinations were performed by the same veterinarian at baseline and 1–3 months of follow-up.

AS was prepared basically according to a previous study[20] and modified by veterinary ophthalmology. In brief, approximately 5–10 mL of blood was drawn from each dog by venipuncture into a blood collection tube without anticoagulant and left at room temperature for 30 min. After clotting, the tubes were centrifuged at 3000 g for 15 min and supernatant was extracted under the sterile condition. Balanced Salt Solution (BSS) was used as buffer solution to dilute serum to 20%, and then prepared serum was aliquoted into sterile eye drop bottles. Finally, the bottles were wrapped with protective sticker to avoid vitamin A degrading due to ultraviolet light. All the owners were indicated to keep one bottle for use in a refrigerator at 4°C, and other bottles were stored in a freezer at −20°C. In the period of study, the owners were informed to continue the conventional treatment and supplement it with the use of AS eye drops four times a day.

Statistical analysis was performed using Statistical Product and Service Solutions version 20 (SPSS Taiwan Corp, Taipei, Taiwan). Dogs baseline characteristics and measurements were presented as mean ± SD. The Wilcoxon sign rank test was performed to compare the change in STT, TBUT, FL staining score and RB staining score between baseline and each time point. In addition, all parameters were stratified by their STT value (STT < 5 and 5–10 mm/min), sex (female and male), and age (< 9 and > 9 years). The Mann-Whitney U test was used to evaluate the statistical significance of the difference between STT value, sex, and age subgroup. Significance was defined as $P < 0.05$.

RESULTS

Medical records of 10 dogs were reviewed in this study. Characteristics of patients and baseline ocular surface parameters in 18 eyes are shown in Table 1. Five dogs were female (one intact and four spayed) and the others were male (one intact and four castrated). There were two Maltese, two Yorkshire Terrier, and one each of Miniature Schnauzer, West Highland White Terrier, Beagle, English Bulldog, Miniature pinscher, American Cocker Spaniel, respectively. Average age was 9.2 ± 2.7 years old (range, 6–15 years), and average body weight was 8.8 ± 7.5 kg (range, 3–26.2 kg). Among 10 dogs, only Miniature Pinscher (OS) and American Cocker Spaniel (OD) had unilateral cKCS.

A total of 18 eyes were analyzed. At baseline, the mean STT was 6.5 ± 3.8 mm/min, the mean TBUT was 5.6 ± 2.9 s, and mean FL and RB staining scores were 4.2 ± 2.8 and 6.3 ± 2.0, respectively. The comparison of pre-treatment and post-treatment is shown in Table 2. After application of AS eye drops, the mean STT values at each time point were not improved compared to that at baseline ($P = 0.977$, $P = 0.849$, and $P = 0.972$, respectively). However, TBUT was significantly increased in each following time point. At the first, second, and third months, 94.4% (17/18), 100% (18/18) and 94.4% (17/18) eyes showed improvement, respectively. Besides, FL and RB staining score were significantly diminished in each following time point. For FL staining score, more than 80% of eyes in each month were considered as advancement. For RB staining score, 44.4% (8/18), 61.1% (11/18) and 88.9% (16/18) of eyes indicated gradual improvement at the first, second, and third month, respectively. In clinical signs, 77.8% (14/18) of eyes had decreased mucopurulent ocular discharges and 38.9% (7/18) got wet.

Table 1. Characteristics of Patients and Baseline Ocular Surface Parameters in 18 Eyes.

Total Dogs (Eyes)	10 (18)
Sex	
Male (eyes)	5 (8)
Female (eyes)	5 (10)
Age (years)	9.2 ± 2.7
Body weight (kg)	8.8 ± 7.5
STT (mm/min)	6.5 ± 3.8
TBUT (s)	5.6 ± 2.9
FL score	4.2 ± 2.8
RB score	6.3 ± 2.0

Notes: Characteristics of patients ($n = 10$) and baseline parameters ($n = 18$) were presented as mean ± SD. STT, Schirmer tear test; TBUT, tear film breakup time; FL score, fluorescein staining score; RB score, Rose Bengal staining score.

In addition, 18 eyes were stratified by the STT value, sex, or age status (Table 3). Firstly, there were seven eyes in the STT < 5 mm/min subgroup and 11 eyes in the STT 5–10 mm/min subgroup. The mean TBUT, FL staining score, and RB staining score did not demonstrate significant difference between both subgroups at any time point (Table 4). Secondly, there were eight eyes in the male subgroup and 10 eyes in the female subgroup. The mean STT, TBUT, FL staining score, and RB staining score also showed no difference between both subgroups at any time point (Table 5). Lastly, there were eight eyes in the < 9 years old subgroup and 10 eyes in the > 9 years old subgroup. Even though the mean STT and FL staining score did not demonstrate differences, mean TBUT and RB staining score showed

Table 2. Comparison of Examination at Baseline and at Follow-up with AS Treatment.

	STT			TBUT		
	Values	***N* (18)**	**%**	**Values**	***N* (18)**	**%**
Baseline	6.5 ± 3.8	—	—	5.6 ± 2.9	—	—
1 month	7.8 ± 5.9	7	38.9	7.5 ± 3.9*	17	94.4
2 months	6.3 ± 3.9	11	61.1	9.1 ± 4.0*	18	100
3 months	6.5 ± 5.0	5	27.8	9.7 ± 5.0*	17	94.4
	FL Score			**RB Score**		
	Values	***N* (16)**	**%**	**Values**	***N* (18)**	**%**
Baseline	4.2 ± 2.8	—	—	6.3 ± 2.0	—	—
1 month	2.7 ± 2.6*	13	81.3	5.8 ± 2.3*	8	44.4
2 months	2.5 ± 2.6*	13	81.3	5.3 ± 2.5*	11	61.1
3 months	2.2 ± 2.1*	14	87.5	4.7 ± 1.8*	16	88.9

Notes: *Significantly ($P < 0.05$) different from the baseline. N is the improved number of cases. Data is represented as mean ± SD.
STT, Schirmer tear test; TBUT, tear film breakup time; FL score, fluorescein staining score; RB score, Rose Bengal staining score.

Table 3. Numbers of Dry Eyes Stratified by the STT Value, Sex and Age Status ($N = 18$ Eyes).

	STT Value (mm/min)		**Sex**		**Age (Years)**	
Group	**< 5**	**5–10**	**Male**	**Female**	**< 9**	**> 9**
N	7	11	8	10	8	10

Note: STT, Schirmer tear test.

significant improvement in the subgroup of less than 9 years old at any time point.

There was no complication, such as severe ocular infections, inflammation, or immune complex deposition, after AS treatment in the whole period of clinical trial.

DISCUSSION

In 1984, Fox *et al.* first described that AS eye drops would be a tear substitute.[13] Since then, many studies have reported that AS eye drops were beneficial for ocular surface disorders.[10,12,14,21] There are several epitheliotrophic factors in tears that are advantageous to ocular surface epithelium including epidermal growth

Table 4. Comparison of Indexes Between STT < 5 and STT 5–10 mm/min at Each Time Point in STT Value Group.

	STT			TBUT		
	STT < 5	**STT 5–10**	***P* Value**	**STT < 5**	**STT 5–10**	***P* Value**
Baseline	2.5 ± 1.7	9.0 ± 2.1	**< 0.001**	4.8 ± 2.7	6.1 ± 3.0	0.364
1 month	6.2 ± 7.1	8.8 ± 5.2	0.144	7.0 ± 4.1	7.9 ± 3.5	0.556
2 months	4.0 ± 3.4	7.9 ± 3.6	**0.040**	8.9 ± 3.5	9.2 ± 4.4	0.964
3 months	3.0 ± 3.6	8.8 ± 4.5	**0.005**	9.3 ± 4.0	10.0 ± 5.6	0.821
	FL Score			**RB Score**		
	STT < 5	**STT 5–10**	***P* Value**	**STT < 5**	**STT 5–10**	***P* Value**
Baseline	4.4 ± 2.7	4.1 ± 3.0	0.964	6.5 ± 1.6	6.2 ± 2.2	0.747
1 month	3.4 ± 3.4	2.2 ± 2.1	0.550	6.0 ± 2.0	5.7 ± 2.5	0.614
2 months	3.1 ± 3.0	2.0 ± 2.3	0.427	5.2 ± 2.2	5.4 ± 2.8	0.927
3 months	3.2 ± 2.6	1.5 ± 1.5	0.125	4.8 ± 1.2	4.7 ± 2.2	0.890

Notes: Data is represented as mean ± SD.
STT, Schirmer tear test; TBUT, tear film breakup time; FL score, fluorescein staining score; RB score, Rose Bengal staining score.

Table 5. Comparison of Indexes Between Male and Female at Each Time Point in Sex Group.

	STT			TBUT		
	Male	**Female**	***P* Value**	**Male**	**Female**	***P* Value**
Baseline	7.8 ± 3.9	5.5 ± 3.5	0.226	4.6 ± 2.8	6.3 ± 2.9	0.229
1 month	8.1 ± 6.7	7.6 ± 5.6	0.929	5.8 ± 2.8	8.9 ± 3.8	0.076
2 months	7.6 ± 3.7	5.4 ± 4.0	0.210	9.0 ± 5.0	9.2 ± 3.3	0.689
3 months	7.3 ± 5.6	5.9 ± 4.7	0.653	9.9 ± 5.6	9.5 ± 4.7	0.929
	FL Score			**RB Score**		
	Male	**Female**	***P* Value**	**Male**	**Female**	***P* Value**
Baseline	4.8 ± 1.7	3.8 ± 3.5	0.244	6.7 ± 2.4	6.1 ± 1.6	0.470
1 month	3.1 ± 2.6	2.4 ± 2.7	0.443	6.6 ± 2.4	5.2 ± 2.0	0.225
2 months	3.0 ± 2.6	2.1 ± 2.6	0.410	6.5 ± 2.6	4.5 ± 2.1	0.098
3 months	2.3 ± 1.7	2.1 ± 2.4	0.494	5.6 ± 1.9	4.1 ± 1.5	0.057

Notes: Data is represented as mean ± SD.
STT, Schirmer tear test; TBUT, tear film breakup time; FL score, fluorescein staining score; RB score, Rose Bengal staining score.

factor (EGF), transforming growth factor β (TGF-β), hepatocyte growth factor (HGF), vitamin A, and fibronectin.[14] In addition to tears, above factors exist in serum as well.[14,15] In human ophthalmology, AS eye drops were applied to the KCS patients that were refractory to conventional treatment. However, the dogs with cKCS usually also had severe corneal disease (corneal pigmentation, vascularization and ulceration) when the owner consulted for medical advice. Based on the above theory, AS eye drops might provide necessary nutrition for corneal and conjunctival epithelium, even in the cases with ocular disorders. As far as we know, AS eye drops are applied to treat severe corneal ulcer in veterinary clinical practices. This study is the first trial to determine the efficacy and safety of 20% AS eye drops for cKCS.

As in many human researches, this study shows that the mean TBUT, FL staining score, and RB staining score were significantly improved after treatment of AS eye drops. Significant relief symptoms were also observed such as decreased mucopurulent ocular discharges and increased ocular surface moisture. However,

Table 6. Comparison of Indexes Between < 9 and > 9 Years at Each Time Point in Age Group.

	STT			TBUT		
	Age < 9	**Age > 9**	***P* Value**	**Age < 9**	**Age > 9**	***P* Value**
Baseline	6.6 ± 3.2	6.5 ± 4.3	0.823	7.7 ± 3.0	3.8 ± 1.3	0.033*
1 month	7.2 ± 5.4	8.3 ± 6.6	0.754	10.0 ± 3.7	5.6 ± 2.2	0.021*
2 months	7.5 ± 4.1	5.5 ± 3.8	0.303	11.8 ± 3.7	6.9 ± 2.9	0.010*
3 months	8.1 ± 6.0	5.3 ± 3.9	0.323	12.5 ± 4.7	7.5 ± 4.1	0.016*
	FL Score			**RB Score**		
	Age < 9	**Age > 9**	***P* Value**	**Age < 9**	**Age > 9**	***P* Value**
Baseline	2.8 ± 2.8	5.4 ± 2.4	0.066	5.1 ± 1.8	7.4 ± 1.5	0.019*
1 month	1.8 ± 3.0	3.4 ± 2.2	0.095	4.7 ± 2.3	6.7 ± 1.9	0.043*
2 months	1.7 ± 3.0	3.1 ± 2.1	0.099	3.7 ± 1.9	6.7 ± 2.2	0.012*
3 months	1.7 ± 2.8	2.6 ± 1.3	0.083	3.2 ± 1.4	6.0 ± 1.1	0.002*

Notes: *Statistically significant at $P < 0.05$. Data is represented as mean ± SD.
STT, Schirmer tear test; TBUT, tear film breakup time; FL score, fluorescein staining score; RB score, Rose Bengal staining score.

the result of RB staining score in this study is different from that in human research. Liu *et al.*[12] reported that the mean RB staining score at baseline in humans with the Sjögren's syndrome dry eye was 2.6 and decreasing score was noted after treatment with 20% AS eye drops for at least more than 3 months.[11,22–24] On the contrary, the RB staining score was apparently lessened after treatment for 1 month, even though the original score was 6.3 in this study. The causes resulting in the differences between humans and dogs might be related to species, underlying cause and severity of dry eye, duration from the onset of disease to the treatment, and frequency of treatment.

Additionally, our study compared the parameters which were stratified by STT value (STT < 5 and 5–10 mm/min), sex (female and male), or age (< 9 and > 9 years). In STT value and sex group, all examinations did not differ significantly between both subgroups at any time point, which suggested that the severity of cKCS and sex would not be factors influencing the efficacy of AS eye drops. As for the mean age of 9.2 years old, we chose 9 as the cut-off point to separate younger and older dogs. Surprisingly, the mean TBUT and RB staining score in $<$ 9-year-old dogs showed better results than $>$ 9-year-old dogs before and after treatment, indicating that dogs younger than 9 years old had better tear stability and lower vital staining scores of ocular surface.

In the period of experiment, all dogs could tolerate AS eye drops with no adverse effect. Complications such as severe ocular infections, inflammation and immune complex deposition were not observed.[15,25] Because there was no preserved method for AS eye drops in human ophthalmology, the potential risk of ocular infection might increase in patients when AS eye drops were stocked in contaminated bottles. In order to minimize the possibility of infection after using AS eye drops, there are some cautions and guidelines, such as preparing under sterile condition, instructing the owner to avoid touch eyes and hair to bottle tip, and discarding the eye drops after thawing for 1 week no matter what contamination happened or not.

Even in human ophthalmology, the optimal concentration and usage of AS eye drops are inconclusive.[26] The reported concentration of AS eye drops ranged from 20% to 100% in different studies.[15,26] In this study, 20% AS eye drops was considered in a variety of aspects. First, Liu *et al.*[20] showed that 12.5–25% dilute serum had the best proliferation to cells, which is more beneficial to ocular surface health. Second, high level TGF-B showed the anti-proliferative effect, which means interfering wound healing of the corneal epithelium. The concentration of TGF-B in non-diluted serum was five times higher than that in tears.[27] Third, the lower concentration had a chance to prevent some side effects, such as corneal immunoglobulin deposits.[25] Last, the use of more diluted AS could decrease the frequency of venipuncture and increase owners' therapeutic compliance. Therefore, we chose the 20% dilute serum as the concentration of AS eye drops in this study.

In conclusion, AS eye drops seemed to be effective and safe for improving tear film stability (TBUT), ocular surface health (FL and RB staining scores) and main clinical symptoms (mucopurulent ocular discharge), especially in younger dogs (< 9 years). We suggested that AS eye drops should be used to supply growth factors, vitamin A, fibronectin, and other cytokines in dogs with cKCS.

REFERENCES

1. Giuliano EA, Diseases and surgery of the canine lacrimal secretory system, in Gelatt KN, Gilger BC, Kern TJ (eds.), *Veterinary Ophthalmology*, 5th edn., John Wiley & Sons, Ames, Iowa, pp. 912–944, 2013.
2. Williams DL, Immunopathogenesis of keratoconjunctivitis sicca in the dog, *Vet Clin North Am Small Anim Pract* **38**:251–268, 2008.
3. Carter R, Colitz CMH, The causes, diagnosis, and treatment of canine keratoconjunctivitis sicca, *Vet Med* **97**:683–694, 2002.
4. Helper LC, The tear film in the dog. Causes and treatment of diseases associated with overproduction and underproduction of tears, *Anim Eye Res* **15**:5–11, 1996.
5. Turner SM, Nind F, Complicated keratoconjunctivitis sicca, in *Small Animal Ophthalmology*, 1st edn., Elsevier, Edinburgh, pp. 93–101, 2008.
6. Kaswan RL, Salisbury M-A, Ward DA, Spontaneous canine keratoconjunctivitis sicca. A useful model for human keratoconjunctivitis sicca: Treatment with cyclosporine eye drops, *Arch Ophthalmol* **107**:1210–1216, 1989.
7. Moore CP, McHugh JB, Thorne JG, Phillips TE, Effect of cyclosporine on conjunctival mucin in a canine keratoconjunctivitis sicca model, *Invest Ophthalmol Vis Sci* **42**:653–659, 2001.
8. Morgan RV, Abrams KL, Topical administration of cyclosporine for treatment of keratoconjunctivitis sicca in dogs, *J Am Vet Med Assoc* **199**:1043–1046, 1991.
9. Olivero DK, Davidson MG, English RV, Nasisse MP, Jamieson VE, Gerig TM, Clinical evaluation of 1% cyclosporine for topical treatment of keratoconjunctivitis sicca in dogs, *J Am Vet Med Assoc* **199**:1039–1042, 1991.
10. Celebi AR, Ulusoy C, Mirza GE, The efficacy of autologous serum eye drops for severe dry eye syndrome: A randomized double-blind crossover study, *Graefes Arch for Clin Exp Ophthalmol* **252**:619–626, 2014.
11. Lee GA, Chen SX, Autologous serum in the management of recalcitrant dry eye syndrome, *Clin Exp Ophthalmol* **36**:119–122, 2008.

12. Liu Y, Hirayama M, Cui X, Connell S, Kawakita T, Tsubota K, Effectiveness of autologous serum dye drops combined with punctal plugs for the treatment of Sjögren syndrome-related dry eye, *Cornea* **34**:1214–1220, 2015.
13. Fox RI, Chan R, Michelson JB, Belmont JB, Michelson PE, Beneficial effect of artificial tears made with autologous serum in patients with keratoconjunctivitis sicca, *Arthritis Rheum* **27**:459–461, 1984.
14. Tsubota K, Goto E, Fujita H, Ono M, Inoue H, Saito I, Shimmura S, Treatment of dry eye by autologous serum application in Sjögren's syndrome, *Br J Ophthalmol* **83**:390–395, 1999.
15. Geerling G, Maclennan S, Hartwig D, Autologous serum eye drops for ocular surface disorders, *Br J Ophthalmol* **88**:1467–1474, 2004.
16. del Castillo JM, de la Casa JM, Sardiña RC, Fernández RM, Feijoo JG, Gómez AC, Rodero MM, Sánchez JG, Treatment of recurrent corneal erosions using autologous serum, *Cornea* **21**:781–783, 2002.
17. Jeng BH, Dupps WJ Jr, Autologous serum 50% eyedrops in the treatment of persistent corneal epithelial defects, *Cornea* **28**:1104–1108, 2009.
18. Matsumoto Y, Dogru M, Goto E, Ohashi Y, Kojima T, Ishida R, Tsubota K, Autologous serum application in the treatment of neurotrophic keratopathy, *Ophthalmology* **111**:1115–1120, 2004.
19. Tsubota K, Goto E, Shimmura S, Shimazaki J, Treatment of persistent corneal epithelial defect by autologous serum application, *Ophthalmology* **106**:1984–1989, 1999.
20. Liu L, Hartwig D, Harloff S, Herminghaus P, Wedel T, Geerling G, An optimised protocol for the production of autologous serum eyedrops, *Graefes Arch Clin Exp Ophthalmol* **243**:706–714, 2005.
21. Kojima T, Ishida R, Dogru M, Dogru M, Goto E, Matsumoto Y, Kaido M, Tsubota K, The effect of autologous serum eyedrops in the treatment of severe dry eye disease: A prospective randomized case-control study, *Am J Ophthalmol* **139**:242–246, 2005.
22. Takamura E, Shinozaki K, Hata H, Yukari J, Hori S, Efficacy of autologous serum treatment in patients with severe dry eye, *Adv Exp Med Biol* **506**:1247–1250, 2002.
23. Tananuvat N, Daniell M, Sullivan LJ, Yi Q, McKelvie P, McCarty DJ, Taylor HR, Controlled study of the use of autologous serum in dry eye patients, *Cornea* **20**:802–806, 2001.
24. Ziakas NG, Boboridis KG, Terzidou C, Naoumidi TL, Mikropoulos D, Georgiadou EN, Georgiadus NS, Long-term follow up of autologous serum treatment for recurrent corneal erosions, *Clin Exp Ophthalmol* **38**:683–687, 2010.
25. McDonnell PJ, Schanzlin DJ, Rao NA, Immunoglobulin deposition in the cornea after application of autologous serum, *Arch Ophthalmol* **106**:1423–1425, 1988.
26. Cho YK, Huang W, Kim GY, Lim BS, Comparison of autologous serum eye drops with different diluents, *Curr Eye Res* **38**:9–17, 2013.
27. Gupta A, Monroy D, Ji Z, Yoshino K, Huang A, Pflugfelder SC, Transforming growth factor beta-1 and beta-2 in human tear fluid, *Curr Eye Res* **15**:605–614, 1996.