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Modification of Electro-Ejaculation Technique to Minimise Discomfort during Semen Collection in Bulls

Falah Baiee[1,3], Wahid Haron[1,*], Rosnina Yusoff[1], Ariff Omar,[2] Nurhusien Yimer[1], Salman Hammadi[1], Tarig Ahmedeltayeb[1], Asmatullah Kaka[4]

[1]*Department of Veterinary Clinical Studies, Faculty of Veterinary Medicine, Universiti Putra Malaysia, UPM 43400 Serdang, Selangor, Malaysia*

[2]*Department of Veterinary Pre-Clinical Studies, Faculty of Veterinary Medicine, Universiti Putra Malaysia, UPM 43400 Serdang, Selangor, Malaysia*

[3]*Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Kufa, Najaf, Iraq*

[4]*Department of Animal Reproduction, Faculty of Animal Husbandry and Veterinary Science, Sindh Agriculture University, Pakistan*

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Authors' Contribution
FB, WH, RY, AO and NY presented the conception and design of the study. FB, SH, TA and AK acquire data. FB and AO analysed the data and WH, FB and RY interpreted it. FB drafted the manuscript.

Key words
Electro-ejaculation, Discomfort sign, Semen collection, Bull, Semen evaluation.

ABSTRACT

The aim of this study was to reduce acute discomfortness experienced in bulls during semen collection by electro ejaculation method. The normal electro ejaculation method of semen collection (Method I) was compared to a modified method involving three stages of graduated electrical stimulation (Method II) in four crossbred bulls. The results showed that intensive muscle spasm, bull struggling and arc back were reduced ($p < 0.05$), as well as the time of penile protrusion ($p = 0.003$) and semen emission ($p = 0.084$) were improved using Method II than Method I. However, the total time taken for semen collection was the same in both methods. Also, there were no significant differences in semen parameters such as sperm volume, motility, morphology, viability, and concentration. In conclusion, gradation of electrical stimulation into three stages (our modified Method II) could help to ease the collection of semen samples from bulls with minimum discomfort signs. Furthermore, the modified method is also recommended to use for other animals, in particular, the wild animals.

INTRODUCTION

In general, semen samples can be collected by one of these methods: artificial vagina (AV) (Palmer, 2016; Barszcz *et al*., 2012), internal artificial vagina (IAV) (Barth *et al*., 2004), electro-ejaculator (EE) (Hill *et al*., 1956), transrectal massage (RM) (Palmer *et al*., 2005; Sarsaifi *et al*., 2013), and transrectal ultrasound-guided massage (TUM) (Santiago-Moreno *et al*., 2013). In fact, AV and IAV are regarded as methods that produce semen samples with highest sperm concentration due to their nearness to the natural breeding, in which bulls willingly donate the semen. However, use of AV requires trained bulls and a dummy or mount cow (Palmer, 2005), while disease transmission, lame cow, breeding crates, trained bulls remain limitation factors of the use of IAV (Barth *et al*., 2004). Electro-ejaculator resembles a very efficient procedure for semen collection from wild and domestic animals. Furthermore, this procedure is easy, safe to apply, improves reliability in obtaining sample and requires minimal facilities (Palmer, 2005). Characteristics of semen that was collected using EE method was better than RM method (Sarsaifi *et al*., 2013, 2015), but not as of high quality as the semen that was collected using AV (Álvarez *et al*., 2012; Jiménez-Rabadán *et al*., 2012). Electrical stimulation is induced via rectal probe to stimulate nerves and accessory sex glands to emit semen. At the time of ejaculation, the peristalsis of the ductus deferens and closure of the internal urethral sphincter are caused by sympathetic nerve; at the same time, the parasympathetic impulses cause peristalsis of the urethral muscle and the branch of pudendal nerve cause contraction of the bulbospongiosus to ejaculate the semen (O'Kelly *et al*., 2011). Thus, using EE to stimulate these nerves to emit semen may be associated with pain in particular for human (Ohl, 1993).

Many studies were carried out to elevate and improve the function of EE device. For instance, Palmer

* Corresponding author: wahidh@upm.edu.my; wahidharon@gmail.com
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et al. (2004) recorded that oxytocin can reduce the time of EE required to collect semen from bull. In addition, they revealed that RM in the general area of ampullae, prostate gland and urethra before EE stimulation did not effect on the time of semen collection when compared with control group. Etson *et al*. (2004) revealed that serum cortisol and progesterone concentration were significantly increased after 5 min following EE with or without epidural anaesthesia, longitudinal (conventional) or segmented rectal probe compared to the control group. They also concluded that there was no difference between conventional and segmented probe.

It is difficult to understand pain in animals (Palmer, 2005). However, we can observe it through their action, for example, struggling, muscle spasms and vocalization. In rare cases the animal may lie down or become recumbent. Hence, the objective of this study was to relieve the amount of acute pain that may occur during semen collection using EE, by gradual increase of the electrical stimuli into three stages, so that the animals involved are more relaxed.

MATERIALS AND METHODS

Management of bulls

In this study, the experimental design was reviewed and approved by the Institutional Animal Care and Use Committee (R073/2015) of Universiti Putra Malaysia. Four crossbred bulls: two Sahiwal-Friesian and two Brangus–Simmental, aged from 5 to 6 years old were involved. The weight of the bulls was between 590 to 660 kg. Bulls were kept in separate sheds at the Universiti Putra Malaysia farm, Serdang Malaysia. The four bulls were fed with *Brachiaria decumbens* grass, and given palm kernal cake given at a rate of 2 kg/body weight. Mineral blocks and water were provided *ad libitum* to the bulls.

Preparation of bulls prior to semen collection

Before collection of semen, the bull was directed into a chute and left to rest for 2 to 3 min. Hairs around the prepuce were clipped and then cleaned with an antiseptic solution (ChlorHEX®) followed by washing with tap water and lastly dried using paper towels. Transrectal massage was applied for 30 to 60 sec to evacuate faeces from rectum and to sexually stimulate the bull as well (Palmer, 2005).

Methods of semen collection

An electro-ejaculator (ElectroJac 6; Neogen® Corporation, Lansing, IM48912; L24290812) was used to collect semen. A rectal probe of EE was then inserted and controlled by one person; another person controlled an EE remote control and recorded the observation appearing on the animal during collection. The third person handled the collection cone and collected semen from the bull. The EE was set on automatic mode; one circuit contained a series of 40 cycles, with each cycle delivering a slightly higher intensity voltage. Each cycle lasted for two s followed by two s pause; which meant, for example, if bull ejaculated at cycle 30, so the duration of time was 120 s. The rectal probe was 51mm in diameter, and 330 mm in length with three electrodes distributed longitudinally and ventrally.

A total of 24 semen samples were collected, 12 each by either Method I or II. For Method I, after insertion of rectal probe, EE device was switched on and continued until ejaculation. In Method II, there were three stages depending on the number of electrical impulses per stage. In the first stage, the EE was switched on increasing the electrical stimulus from zero until four (1st stage took 16 s), and at that point, EE was switched off, then immediately switched on for the start of the second stage. The process was repeated in the second stage until the electrical stimuli was at eight (2nd stage took 32 s). EE was then switched off and switched on again to start the final 3rd stage; it was started from zero and continued until semen is emitted.

Discomfort signs

For the two methods mentioned above, all discomfort signs were recorded and graded. The signs include all irregular movement of hind limbs, kicking, moving back and forth, muscle spasm at the thighs and abdominal region, arc back, and recumbent (Table I). All these observations were recorded by the same person throughout the experiment. Furthermore, time of penile protrusion, time of semen emission and number of electrical stimuli were also recorded.

Table I.- Scoring of signs of discomfort in bulls during semen collection using electro-ejaculation.

Categories	Score	
Muscle spasm*	Moderate	Intense
Animal struggling**	Back and forth	Intense movement
Recumbent	Yes	No
Arc back	Yes	No

*Muscle spasm, contraction of muscles in thighs and abdominal region that appeared during semen collection using EE; **Animal struggling, intense movements of hind limbs, including kicking, trying to move backward and forward during semen collection using EE.

Semen evaluation

Sperm motility and concentration were evaluated using computer-assisted sperm analysis (CASA; Hamilton Thorne Bioscience). Initially, fresh semen was diluted with buffer saline 1:40, in a water bath at 37°C. About 10 µL of semen was placed on a dry warm dual sided sperm

analysis chamber and covered with a cover slip, and then placed in CASA. At least three microscopic fields were recorded from each chamber of dual slide (Yimer *et al*., 2011).

Viability and morphology of sperm were evaluated using eosin-nigrosin staining (Felipe-Pérez *et al*., 2008). For this, 5 µL of semen was mixed with 15 µL of eosin-nigrosin for one min at room temperature. Then, a smear was made and allowed to dry on a slide warmer (Copens Scientific (M) Sdn. Bhd.) maintained at 40 to 50 °C, and subsequently examined under phase contrast microscope (Nikon Japan, ECLIPSE E200) at 400× magnification. Stained sperm head was classified as dead sperm and unstained sperm head indicated as live. Sperm morphology was also determined where at least 200 sperms were evaluated at 1000×.

Statistical analysis

Data were checked for conformity to a normal distribution. Comparison of the 2 methods of semen collection was done through Fisher's exact test and independent *t*-test using SPSS version 22, (IBM Corporation; Armonk, USA).

RESULTS

In Method I, the EE was switched on using auto-mode and continued until the semen sample was successfully collected. When EE was turned on and the electrical stimulation began, the bull showed excessive muscle spasms and struggling, and these signs increased progressively with increase in electrical stimulation. Intensive muscle spasm decreased significantly from 1st stage to 2nd stage in Method II (Fig. 1A), and this spasm was not observed at 3rd stage. On the other hand, moderate muscle spasm increased from 1st stage to 3rd stage. We observed that in Method I and 1st stage of Method II, all the bulls refused the electrical stimulation based on the following signs observed: animal struggling and irregular movements of the hind limbs (Fig. 1B) and arc back (Fig. 2B). However, struggling and arc back were gradually decreased in the 2nd stage and totally disappeared in the 3rd stage of Method II. Moreover, there was also back and forth movement of the left hind limb; this movement was regularly observed with electrical stimulation in the 3rd stage of Method II. In this study, only two bulls became recumbent, and this was recorded in Method I only (Fig. 2A).

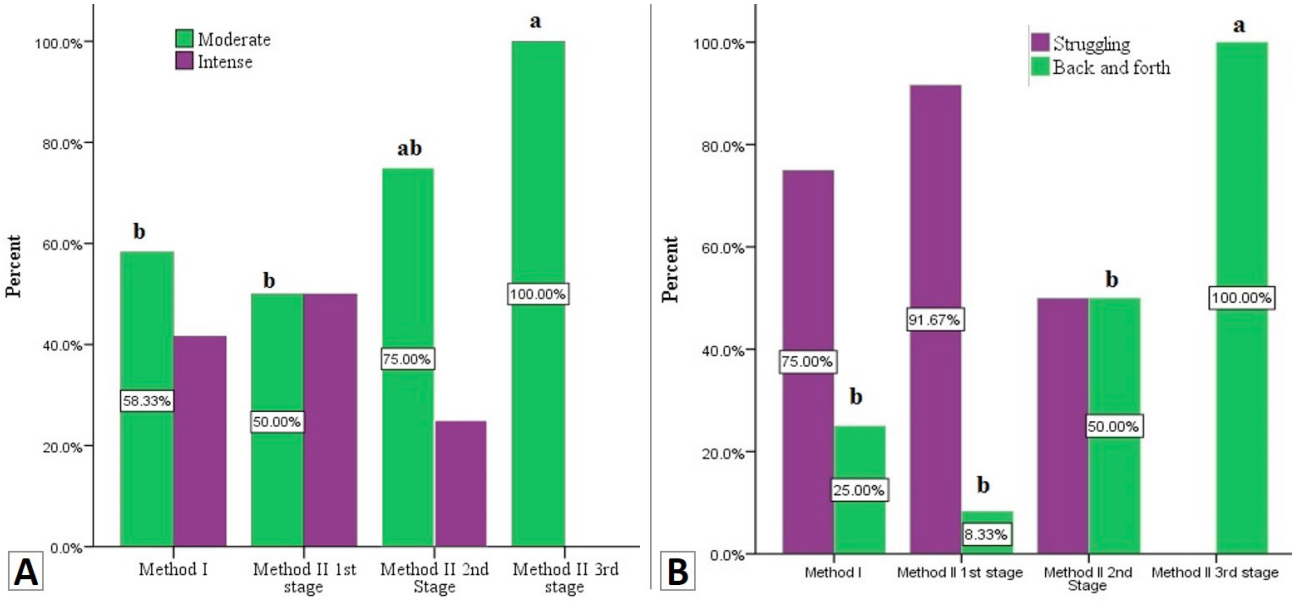

Fig. 1. Muscle spasm (A) and animal struggling (B) in bulls during semen collection by different electro-ejaculation methods. Method I, EE device was switched on and continued until ejaculation; 1st stage of Method II, EE was switched on, increasing the electrical stimulus from zero until four cycles (1st stage took 16 s); 2nd stage of Method II, immediately after 1st stage, EE was switched on for the start of the 2nd stage. The process was repeated in the 2nd stage until the electrical stimuli was at eight cycles (2nd stage took 32 s); 3rd stage of Method II, EE was then switched off and switched on again to start the final 3rd stage, it was started from zero and continued until semen was emitted; Muscle spasm, contraction of muscles in thighs and abdominal region that appeared during semen collection using EE; Animal struggling, all intense movements of hind limbs, including kicking, trying to move backward and forward during semen collection using EE; [ab] values with different superscripts are significantly different among methods; n = 24.

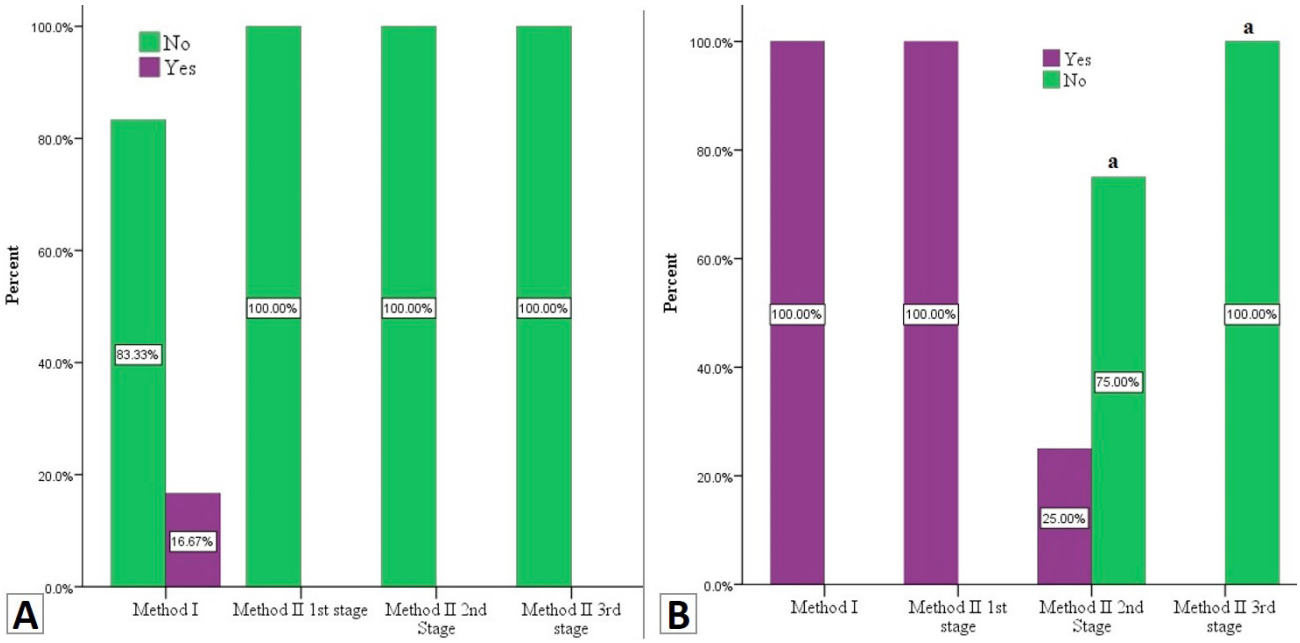

Fig. 2. Recumbent of bulls (A) and arc back in bulls (B) during semen collection by different electro-ejaculation methods. Method I, EE device was switched on and continued until ejaculation; 1st stage of Method II, EE was switched on, increasing the electrical stimulus from zero until four cycles (1st stage took 16 s); 2nd stage of Method II, immediately after 1st stage, EE was switched on for the start of the 2nd stage. The process was repeated in the 2nd stage until the electrical stimuli was at eight cycles (2nd stage took 32 s); 3rd stage of Method II, EE was then switched off and switched on again to start the final 3rd stage, it was started from zero and continued until semen was emitted; [ab] values with different superscripts are significantly different among methods; n = 24.

Table II.- Bulls' response to two electro-ejaculation methods (Mean±SEM).

Items	Method I**	Method II	P value
Time to penile protrusion (s)	77.67 ± 4.10	57.00 ± 4.65	0.003
Time to semen emission (s)	157.33 ± 21.04	109.33 ±16.16	0.08
No. of stimuli required for penile protrusion	19.42 ± 1.03	14.25 ± 1.16	0.003
No. of stimuli required for semen emission	39.33 ± 5.26	27.33 ± 4.04	0.08
Percentage of animals required 1 electrical circuit* (%)	66.70	91.70	0.32
Percentage of animals required 2 electrical circuits (%)	33.30	8.30	0.32
Total time required to collect the semen sample (s)	167.33±21.04	169.25±16.02	0.94

n = 24; *Electrical circuit, electrical stimuli starting from zero to 40 cycles, each cycle continue two s and two s off and the device increases the intensity until reaching the maximum setting 40, then the cycles back to the beginning; **Method I, EE device was switched on, at auto mode, and continued until ejaculation; Method II, is a modified method of EE which contained three stages of electrical stimuli; 1st stage EE was switched on, increasing the electrical stimulus from zero until four cycles (1st stage took 16 s); 2nd Stage of Method II, immediately after 1st stage, EE was switched on for the start of the 2nd stage. The process was repeated in the 2nd stage until the electrical stimuli was at eight cycles (2nd stage took 32 s); 3rd Stage of Method II, EE was then switched off and switched on again to start the final third stage (3rd stage), it was started from zero and continued until semen was emitted. All stages of Method II were at auto mode of EE.

In the present study, the time of penile protrusion and number of electrical stimuli required to cause penile protrusion were significantly reduced in Method II (P = 0.003). Furthermore, the time of semen emission and number of electrical stimuli required for semen emission were also reduced using Method II, but was not significant (P = 0.084). Percentage of electrical circuit was reduced in Method II; however, the total time of collection was slightly increased in Method II (Table II).

Sperm volume, motility and concentration, of fresh semen were higher but not significant in Method II compared with Method I. However, morphology and viability rate were slightly higher in Method I compared to Method II (Table III).

Table III.- Fresh bull semen parameters collected using two different electro-ejaculation methods (Mean ± SEM).

Parameter	Method I	Method II	P Value
Semen volume (mL)	6.98±0.69	8.11±0.96	0.35
Sperm motility (%)	80.58±1.51	80.83±1.30	0.90
Sperm morphology (%)	95.00±0.69	94.50±0.94	0.67
Sperm viability (%)	89.13±0.99	88.92±0.85	0.87
Sperm concentration (10^6/mL)	804.9±47.72	839.6±44.01	0.60

For statistical details, see Table II.

DISCUSSION

The results represented in this study were to compare the effect of Method I and Method II of electro-ejaculator in bulls on the physical reaction and semen quality. The bulls were relaxed during semen collection when electrical stimuli were given in gradation. There were reductions in muscle spasms, struggling and back arching. Regardless, whether bulls experience pain or not during semen collection by EE, the animals tend to struggle, vocalise or attempt to lie down when they are subjected with something which they are unfamiliar with. However, we can surmount this issue by adapting the animal to it. Thus, this study was designed to reduce struggling in bulls during semen collection. In fact, stress or pain that could be inflicted during intramuscular injection, transrectal palpation or any clinical examination was sufficient to boost the level of plasma cortisol of cattle (Etson *et al*., 2004). Whitlock *et al*. (2012) demonstrated that EE stimulation group and insertion of probe into rectum without EE stimulation group led to acute increasing of plasma cortisol compared with the control group. Later, cortisol in the inserted group did not change, while cortisol in EE stimulation group continued increasing significantly in the 20th and 45th min. Substance P is another factor released due to pain (Allen *et al*., 1997). It is a neuropeptide and has a substantial role in response to stress or pain (deVane, 2001). Whitlock *et al*. (2012) revealed that there were no significant differences in the concentration of substance P in bulls following electrical stimulation, insertion of probe without electrical stimulation and control group. This investigation supports our suggestion in the present study.

In Method I, the electrical stimuli were applied until the bull ejaculated and semen collected on the condition that there was no severe struggling or animal become recumbent, *i.e.* the total time of collection started from zero to complete semen collection. In Method II, the total time of semen collection encompassed the stage of semen collection (3rd stage) plus the duration of 1st stage and 2nd stage (16 s and 32 s, respectively); thus, it was thought that Method I should take less time than Method II but the results revealed that there was no significant differences between the two methods in terms of time of semen collection. The reason is very clear if we see the percentage of electrical circuit (Table II). The double of electrical circuit was higher in Method I than in Method II. In Method I, 33.30% of bulls completed one electrical circuit until electrical stimuli 40 and started with another one then semen was emitted, but for Method II, only 8.30% of bulls required two electrical circuits to complete ejaculation (one bull solely required two electrical circuits from 12 ejaculates in that method). These investigations can also explain the differences between number of electrical stimuli required for penile protrusion and number of electrical stimuli required for semen emission in Methods I and II (19.42 ± 1.03; 39.33 ± 5.26) and (14.25 ± 1.16; 27.33 ± 4.04) respectively. In the bulls that required two electrical circuits, the penile protrusion was achieved in one circuit but semen emission occurred in two circuits. The data of number of electrical stimuli required for penile protrusion and for semen emission in Method II was very close to the pervious study that was done on Bali bulls (Sarsaifi *et al*., 2013). They recorded 14.43 ± 0.76 and 23.20 ± 0.97 for the number of electrical stimuli required to penile protrusion and number of electrical stimuli for semen emission respectively. Toosi *et al*. (2013) used a long acting narcoleptic tranquilizer (piportil-L4) to collect semen sample from bison bull using EE. The time for semen collection was between 125 and 145 s depending on the dose administered in each group. This is shorter than what was recorded in the present study. The probable reason is that the previous study used a tranquilizer for semen collection.

Comparison of semen parameters between Method I and II, showed no significant differences. However, there was a slight increase in concentration of sperm and volume of semen in Method II. Sperm concentration was relatively less than in previous studies (Kaka *et al*., 2015; Khumran *et al*., 2015). They used almost the same bulls which they used in the present study in UPM farm. This decreased in semen concentration might be due to bulls getting older (Al-Kanaan *et al*., 2015; Brito *et al*., 2002). In addition, EE stimulates accessory sex glands via electrical pulses, which may lead to decreased concentration of sperm but increased of seminal fluid in the semen sample (Jiménez-Rabadán *et al*., 2012). Furthermore, Rehman *et al*. (2016) reported that the crossbred bulls have less semen quality than purebred bulls. Many studies suggested that a reverse relationship between volume and sperm concentration existed when ejaculates were obtained by EE (Jiménez-

Rabadán *et al*., 2012; Marco-Jiménez *et al*., 2008; Sarsaifi *et al*., 2013). Nevertheless, in this study, there was an increase (but not significant) in volume of semen using Method II (8.11 ± 0.96 mL) compared to Method I (6.98 ± 0.69 mL) and an increase in concentration of semen (not significant) in Method II (839.67 ± 44.01 X 10^6 sperm/mL) compared to Method I (804.92 ± 47.72 X 10^6 sperm/mL). This is probably due to the calmness of bulls when Method II was applied via graduation of electrical stimuli.

CONCLUSION

In conclusion, gradual increase of the electrical stimulation into three stages (Method II) to facilitate semen collection from bulls lead to minimal discomfort signs significantly as compared to normal method (Method I). Although, Method II should take more time than Method I; however, there is no significant differences between methods in terms of time of semen collection. Furthermore, according to the present finding, the modified method is also recommended to use for other animals in particular the wild animals. Since the sample size of the present study was limited; thus, further study is needed with larger sample size to confirm the findings of the present study.

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Statement of conflict of interest

Authors declare no conflict of interests.

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