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RESEARCH ARTICLE

Morphometric and Stereological Assessment of The Bovine Vesicular Gland

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ABSTRACT

The vesicular gland is an important genital gland related to the fertility of bovine. In this study, the morphometric and stereological methods were sought to adapt to the bovine vesicular gland. For this aim, 8 healthy Holstein bull's paired vesicular glands were collected from the slaughterhouse. The weight and the dimensions of the glands were measured before the fixation. After the formalin fixation, all the glands were divided into 3 subgroups for the total volume estimation of the gland, cell counting and the volume estimation of the cell nucleus. The cell counting and the volume estimation of the cell nucleus were performed on the principal cells. The average weight, width, length, height, the volume of the glands, the mean number and nucleus volume of the principal cells were 30.9 g., 2.62 cm, 8.86 cm and 1.86 cm, 29.7 cm^3, 3.58.10^9 and 108 μm^3, respectively. The researchers believe that the findings will contribute to the literature and in particular, facilitate experimental and toxicological researches performed in the future.
Keywords: Cell number, Nucleus volume, Smooth fractionator, Stereology, Vesicular gland

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Sığır Veziküler Bezinin Morfometrik ve Stereolojik Olarak Değerlendirilmesi

ÖZ

Veziküler bez sığır fertilitesinde önemli bir üreme bezidir. Sunulan bu çalışmada sığır veziküler bezinin morfometrik ve stereolojik olarak incelenmesi amaçlanmıştır. Bu amaçla 8 sağlıklı Holstein boğasının çift olan veziküler bezleri mezbahadan toplanmış ve ağırlığı ile boyutları ölçülmüştür. Formaldehit tespitinden sonra tüm bezler bezin toplam hacminin, hücre sayısının ve çekirdek hacminin ölçülmesi için 3 alt gruba ayrılmıştır. Hücre sayımı ve hücre çekirdek hacmi ölçümü bezin en çok bulunan ana hücresi olan principal hücreler üzerinde gerçekleştirilmiştir. Bezin ortalama ağırlığı, eni, uzunluğu, yüksekliği, ortalama hacmi, principal hücrelerin ortalama sayısı ve ortalama çekirdek hacmi sırasıyla 30,9 gr., 2,62 cm, 8,86 cm, 1,86 cm, 29,7 cm^3, 3,58,10^9 ve 108 μm^3 olarak bulunmuştur. Araştırmacılar bu bulguların literature katkı sağlayacağına ve gelecekte yapılacak olan deneysel ve toksikolojik çalışmalara ışık tutacağına inanmaktadır.
Anahtar Kelimeler: Çekirdek hacmi, Hücre sayısı, Smooth parçalama, Stereoloji, Veziküler bez

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INTRODUCTION

The accessory genital glands are fully developed in the bull (Budras et al. 2003). The paired vesicular gland is one of the accessory genital glands (Frandson et al. 2003) and this gland contributes to the bulk of the seminal fluid (Pakurar and Bigbee 2004). The fluid of this gland is the source of energy for the spermatozoon motility (Gartner and Hiatt 2006). The vesicular gland of the bovine is an irregular, lobulated gland surrounded by a fibromuscular capsule. It is more or less solid with narrow branching lumina (Aughey and Frye 2001, Frandson et al. 2003, Marettova and Legath 2010, Rahman et al. 2010). The ducts are separated in the lobules and terminated as elongated and coiled tubules (Cons 1957). The mucosa of these tubules is made of an epithelium and lamina propria surrounded with a muscular layer (Badia et al. 2006, Rahman et al. 2010). The epithelium of these tubules has double layer and contains three different types of cells; principal (formerly called type A), basal (formerly called type B) and dense (formerly called type C) cells. The principal cells are the most abundant main cells, which form an inner lining. These cells are tall, columnar, and have a large oval nucleus (Amselgruber and Feder 1986, Cons 1957) (Figure1).

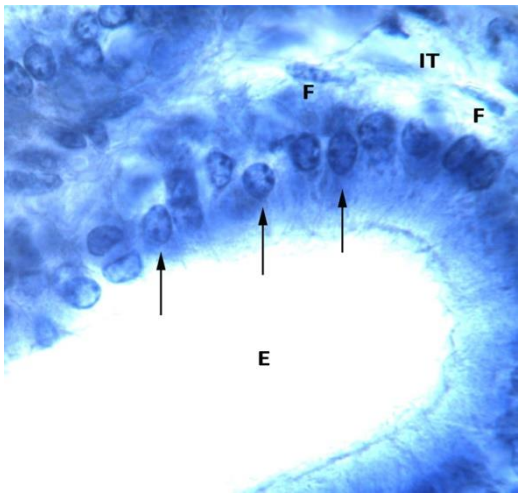

Figure 1: The vesicular gland histology (40 µm thick section, x100 objective). Arrows indicate the principal cells, (E) Endpiece, (F) Fibroblast and, (IT) Interstitial tissue.
Şekil 1: Veziküler bez histolojisi (40 µm'luk kalın kesitler, x100). Oklar principal hücreleri, (E) Endpiece, (F) Fibroblastları ve (IT) Intersitisyel dokuyu işaret etmektedir.

The structure investigation of the organ is important in the morphological and experimental researches (Altunkaynak et al. 2007, Aslan et al. 2006). The changes in the organ structure are directly related to its functional reserve as an indicator of the health status. The stereological methods produced unbiased estimates about the tissue and highly statistically efficient, precise results without any assumptions (Michel and Cruz-Orive 1998, Santos et al. 2009, Sørensen 1989, 1991). The stereological methods could use on the morphological, experimental, toxicological and tumoural studies. The stereological methods are unbiased, precise and independent from the tissue shrinkage (Santos et al. 2009). One of the stereological methods used in this study is the Cavalieri method. This method could easily estimate the total volume on the selected sections obtained from the organ (Gundersen et al., 1988). The second method was the optical fractionator method. It is a precise, unbiased and modern stereological method for the nucleus counting and combines the optical disector with the fractionator method (West et al. 1991). Optical disector is a 3D probe for particle counting in the thick sections (West et al. 1991, Gundersen et al. 1999). The fractionator is a sampling design that samples the organ systematically and randomly (West et al. 1991, Gundersen et al. 1988). However, the smooth fractionator technique is a modification of the fractionator method. This modification has gained increased efficiency to the fractionator method. In order to increase efficiency, the items are arranged (slab, sections, etc.) in symmetrical design with a peak and minimal jumps (Gundersen 2002). The third method is the nucleus volume estimation of the cell. The nucleus volume estimation of an organ is clearly and significantly important for the tumoural grading. The stereological methods could estimate the nucleus volume even in the irregular shaped particles and produced efficient and precise results (Sørensen 1989, 1991). In this study, the volume estimation method was combined with the vertical sectioning procedure (Baddeley et al. 1986, Sørensen 1991).

In the present study, the morphological and stereological evaluations were performed on the

bovine vesicular gland. The stereological methods were sought to adapt to the bovine vesicular gland for the first time according to our knowledge. The obtained findings of this study and the stereological applications may facilitate the future experimental, toxicological and tumoural researches.

MATERIAL and METHODS

In this study, the morphometric and stereological data were obtained from the vesicular gland of the healthy Holstein bulls (2.5-3 years, 650-700kg). The morphometric data were collected from the paired glands of the 8 Holstein bulls. For this aim 16 vesicular glands of 8 animals were obtained from the slaughterhouse. All the glands were weighed (Kern, Balingen-Germany) and the dimensions were measured using vernier calliper before the fixation (Labomar, 304B-01- Turkey).

After the morphometric measurements, all the 16 glands were fixed with the neutral buffered 10% formaldehyde during two weeks. After the fixation, all the glands were divided into three subgroups for explaining the methods clearly. The first group was the glandular volume estimation (n=6) group, the second group was the cell counting (n=5) group and the third group was the cell nucleus volume estimation (n=5) group.

Firstly, the Cavalieri principle (Gundersen et al., 1988), was performed to estimate the mean gland volume. Therefore six fixed glands were systematically sliced into 3 mm intervals with a random start to obtain ~8 slabs per gland. The area of these slabs was measured with 0.3 cm spaced point grid. The calculations per gland were performed by the formula below;

$V = [t \times a/p \times \sum P]$ cm^3,

t; is the slab thickness,

a/p; is the area represented by one point,

$\sum P$; is the total counted points.

In the cell counting (Gundersen, 2002) group, the glands were sliced into 3 mm intervals (from a random start in the first 3mm distance) to obtain ~8 slabs per gland and every third slab was chosen to obtain a fraction of 1/3 (Figure 2/A). The smaller slabs were placed peripherally and the larger ones were placed centrally (Figure 2/B). After the paraffin embedding, the length of the tissue in the paraffin block was measured and separated with a knife into nine equal pieces from a random start in the first interval (Figure 2/C). Every piece was turned 90^0 to the same side, collected in the metal holder and re-embedded into paraffin block (Figure 2/D). This block was sliced into 40 μm sections and the slices were chosen which passed through the centre of the tissues and then staining with the Giemsa's azure eosin methylene blue solution (Figure 2/E). The counting procedure was performed on the nine tissues per gland.

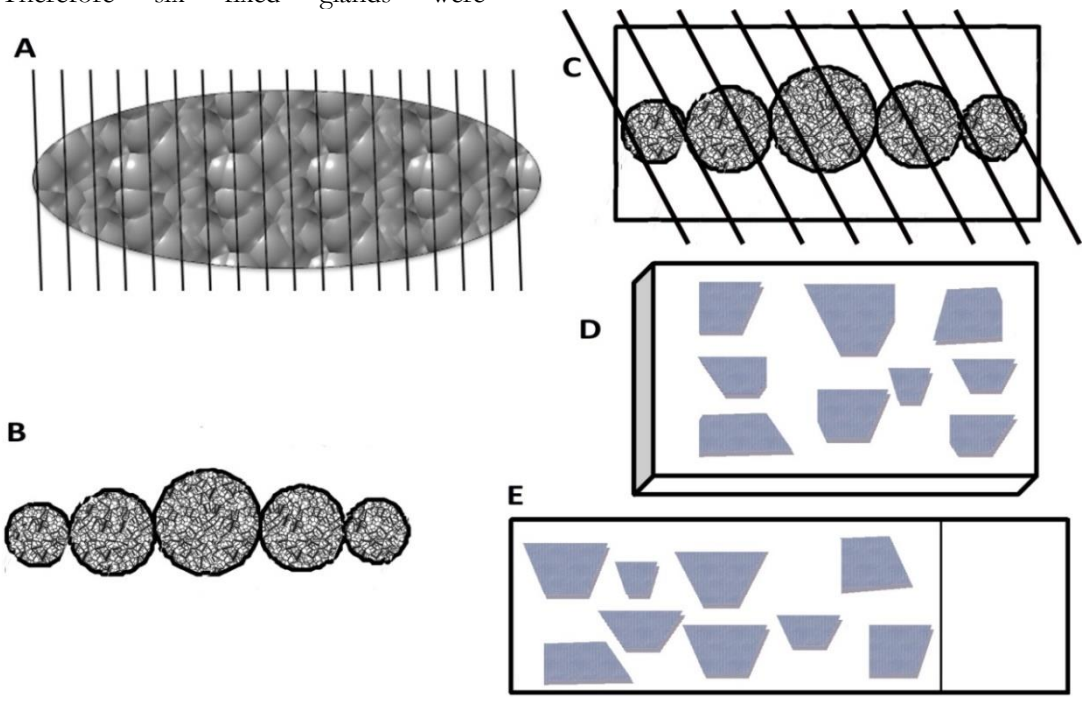

Figure 2: The diagram of the baseline sampling scheme for the cell counting: A; The gland was sectioned from a random start, B; The slabs were arranged according to the smooth fractionator principle and totally embedded in paraffin, C; The length of the tissue was measured and divided D; The pieces were totally embedded in paraffin at the metal holder, E; The paraffin block was sliced for the cell counting.

Şekil 2: Hücre sayımı için örnekleme şeması: A; Bez rastgele bir başlangıçla dilimlendi, B; Dilimler smooth fractionator metoduna göre dizildi, C; Doku uzunluğu ölçülüp, bölündü, D; Parçalar parafine gömüldü, E; Hücre sayımı için blok dilimlendi.

Finally, in the (n=5) nucleus volume of the cell estimation group (Baddeley et al. 1986, Møller et al. 1990, Sørensen 1989, 1991), each gland was sliced into 3 mm intervals and every third slab was chosen. These slabs (with a random start) were re-sliced into 3 mm bars and every 7th bar was chosen. They were rotated around their vertical axis and embedded into the paraffin. The paraffin block was sectioned into 40 µm thickness and the sections were selected which pass through the centre of the tissue.

Stereological Analysis

The number of the principal cell was estimated using a computer loaded Shtereom I software (Oguz et al. 2007), attached to a light microscope with a motorized stage. The thickness of the tissues on the slides was measured by microcator. The researchers sought to count between 100-200 nuclei per gland according to the disector principle (Gundersen et al. 1999). The counting frame area, step lengths along the X and Y axis and the disector height were set at about 76.9 µm², 1250 µm and 10 µm respectively (Figure 3).

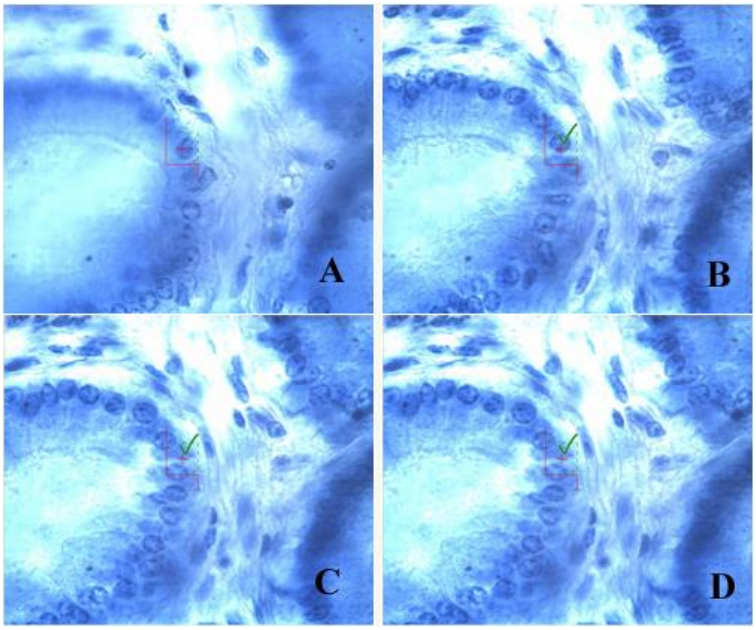

Figure 3: The principal cell counting process with optical disector in the vesicular gland (x100) A; (0 µm), B; (8 µm), C; (13 µm), D; (21 µm).
Şekil 3: Optik disektör sondasıyla veziküler bezde principal hücre sayımı (x100) A; (0 µm), B; (8 µm), C; (13 µm), D; (21 µm).

The total principal cell number was estimated according to the following formula (West et al. 1991).

$N = 1/F_{ssf} \times 1/F_{hsf} \times 1/F_{asf} \times 1/F_{slab} \times \sum Q^-$

F_{ssf}; the sampling fraction of the section,

F_{hsf}; the sampling fraction of the height (the mean section thickness divided by the height of the dissector,

F_{asf}; the sampling fraction of the area (multiplying the step lengths and divided by the area of the frame,

F_{slab}; the converted value to the micron of the tissue length in the paraffin, and this value is divided by the height of the slicing section,

$\sum Q^-$; the counted principal cells.

The coefficient error of the counted nucleus and the mean section thickness (the mean section thickness measured with the microcator per area during counting principal cells on the glass slide) were estimated according to Gundersen's formula (Gundersen et al. 1999):

$$CE(N) = \sqrt{CE^2(Disector) + CE^2(t)}$$

The volume of the principal cell nucleus was estimated on the live image reflected the computers screen. The orientation frame and the test probe were both placed on the monitor. The nucleus that fell inside the orientation frame and intersected with the transparent test probe was evaluated. This transparent test probe also guided the ruler for measuring the nucleus. The ruler was in 15 classes and the total length of the ruler was 35 mm. The final magnification of the cell on the monitor was calculated as x1960. Almost 250 principal cell nuclei were estimated per gland (Figure 4). The formula for the nucleus volume estimation was:

$V_v = \pi/3 \times (L_{15} \times 1000/3.00 \times Magnification)^3 \times l_0^3$ (Sorensen,1991).

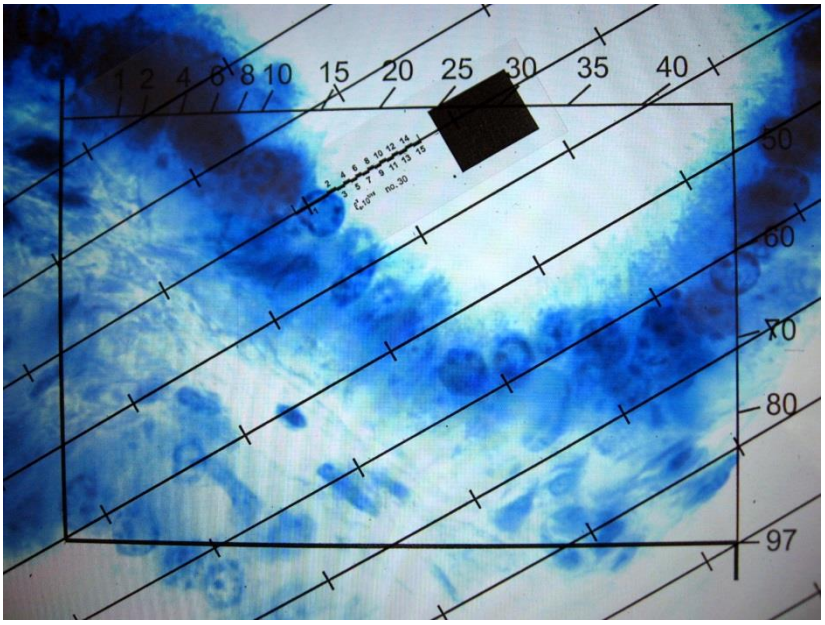

Figure 4: The nucleus volume estimation of the principal cells with ruler in the vesicular gland.
Şekil 4: Cetvel vasıtasıyla veziküler bez principal hücrelerinde çekirdek hacmi hesaplanması.

RESULTS

The mean weight of the gland was 30.9 gr. The mean width, length and height of the glands were 2.62 cm *(SD=0.30)*, 8.86 cm *(SD=1.18)* and 1.86 cm *(SD=0.32),* respectively (Table 1).

Tablo 1. Sol ve sağ veziküler bezler hakkında morfometrik veriler.
Table 1. The morphometric data about the left and right vesicular glands.

No.	Gland	Width	Length	Height	Gland	Width	Length	Height
1	left	2.56 cm	7.87 cm	1.47 cm	right	2.13 cm	7.68 cm	1.50 cm
2	left	2.67 cm	7.60 cm	1.80 cm	right	2.55 cm	7.24 cm	1.83 cm
3	left	2.45 cm	8.00 cm	1.50 cm	right	2.24 cm	8.30 cm	1.50 cm
4	left	2.83 cm	10.1 cm	2.07 cm	right	3.00 cm	10.6 cm	2.22 cm
5	left	2.60 cm	8.00 cm	2.20 cm	right	2.39 cm	8.86 cm	1.80 cm
6	left	3.37 cm	9.08 cm	2.76 cm	right	2.88 cm	9.05 cm	1.60 cm
7	left	2.51 cm	10.6 cm	1.96 cm	right	2.56 cm	10.6 cm	1.90 cm
8	left	2.78 cm	9.51 cm	1.88 cm	right	2.43 cm	8.63 cm	1.79 cm
Mean± SD		2.72 cm±0.29	8.85 cm±1.14	1.95 cm±0.41		2.52 cm±0.30	8.87 cm±1.22	1.77 cm±0.24

All the vesicular glands examined displayed normal histology, and the epithelial cells were visualized at every depth in all the sections. The mean volume of the gland was found as 29.7 cm^3 *(CE=0.09)* (Table 2). The estimated mean amount of the principal cell in the vesicular gland was $3.58.10^9$ and the mean coefficient of error (CE) was 0.09 (Table 2). The mean volume of the principal cell nucleus was 108 μm^3 (Table 2). The shrinkage of the sliced tissue on the glass slide where the cells were counted and the mean final thickness of the slice was 46.5 % and 21.4 µm (*SD=1.5*), respectively.

Table 2. The mean volume of the vesicular gland, principal cell number and the nucleus volume.
Tablo 2. Veziküler bezin ortalama değerleri, principal hücre sayısı ve çekirdek hacmi

No.	The Mean Volume of the Gland	CE Value	The Number of the Principal Cell in the Gland	CE Value	The Nucleus Volume of the Principal Cell in the Gland
1	37.9 cm^3	0.065	$4.28.10^9$	0.09	97 μm^3
2	24.8 cm^3	0.083	$3.39.10^9$	0.10	110 μm^3
3	26.7 cm^3	0.130	$3.89.10^9$	0.09	103 μm^3
4	36.5 cm^3	0.110	$2.83.10^9$	0.10	111 μm^3
5	27.0 cm^3	0.062	$3.50.10^9$	0.09	120 μm^3
6	25.5 cm^3	0.089	-	-	-
Mean± SD	29.7 cm^3±5.9	0.090	$3.58.10^9$±$5.44.10^8$	0.09±0.005	108 μm^3±8.7

DISCUSSION

In the present study the weight, volume, external dimensions, cell number and the nucleus volume of the principal cell in the vesicular gland were estimated. This genital gland of the bulls was investigated by several researchers, previously. In one of this, the vesicular gland of Bangladesh's indigenous bull was investigated. According to the morphological measurements, the weight of the gland was different from the Holstein bulls in this study. The Holstein bull's vesicular gland (~30 gr) was heavier than the indigenous bull (~20 gr). However, the other parameters such as length and width were the same except thickness. The Holstein bull's vesicular gland (~1.9 cm) was thicker than the indigenous bull (~1.5 cm). These differences may the reflection of the beef aimed breeding of Holstein bulls. However, Budras et al. (2003) and Dyce et al. (1999) reported different findings from the Holstein and indigenous bull. They were estimated the gland mean length was between 10 or 20 cm and width was 3 or 5 cm. However, the length (~9 cm) and the width (~2.6 cm) of the Holstein bull's vesicular gland were still in the range of the Rahman et al. (2010) and Dursun's (1998) reports.

Studies on the male accessory glands are generally concentrated on its morphometric and histological features (Badia et al. 2006, Budras et al. 2003, Chandolia et al. 1997, Frandson et al. 2003, Macmillan and Hafs 1969). In the literature, the stereological studies are not adequate. A study on the mice vesicular gland performed using stereological methods was revealed the harmful effects of alcohol usage (Gomes et al. 2002). The chronic alcohol intake has negative effects on the secretory process of the vesicular gland and causes morphological alterations on the epithelial cells. Besides the toxicological studies, in experimental research the triggering effect of the melatonin hormone on the reproductive activity of the inactive rams in the non-breeding season was investigated evaluating the principal cell height and the nucleus diameter (Mokhtar et al. 2016). The stereological methods were performed to the Holstein bull's bulbourethral gland, previously (Akosman et al. 2013). According to this research, there was $3.22.10^9$ principal cell in the bulbourethral gland and the nucleus volume of this cell was 59.1 μm^3. The epithelial cell number in the bulbourethral gland of the rabbit and guinea pig was also evaluated with the stereological methods (Vasquez and del Sol 2014). The development of the mice prostate gland (Singh et al. 1999) and the tumoural grading also investigated with the stereological methods (Santamaria et al. 2016). In another research, seasonal structural changes of the donkey vesicular gland were observed with estimating the nuclear/cell ratio and the interstitial/ glandular tissue ratio (Abou-Elhamd et al. 2020).

In this study, the tissues were embedded in paraffin. The paraffin embedding creates an almost 50% shrinkage in volume (Dorph-Petersen et al. 2001). In the present study, this value was estimated as 46.5%. When the square of the coefficient error divided by the square of the coefficient of variance, the result should between the values of the $0.25<X<0.5$. In this study, the obtained ratio was 0.36, which is in the acceptable range (Lodrup et al. 2008, West et al. 1991).

In conclusion, experimental investigations on the vesicular gland generally rely on calculating the height of the tubular epithelium and the tubule diameter (Archana et al. 2009). Besides the measurements, estimating the number and the nucleus volume of the

glandular cells using stereological methods will make a major contribution to the accuracy of the recent research.

Conflict of Interest: The authors declare that they have no conflict of interest.

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