Ovarian Follicular Dynamics in Caqueteño Creole Cattle Breed at the Colombian Amazon Piedmont

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Abstract: Caqueteño creole cattle breed is part of the eight Colombian creole cattle breeds. These breeds are characterized by having great adaptive potential to the environmental conditions in which they are found and allowing them greater productive efficiency. Caqueteño creole cattle is a breed in danger of extinction according to parameters referenced by FAO. since it is a population of cattle made up of fewer than 350 individuals. This situation suggests the development of multiple research activities with a reproductive focus to efficiently implement biotechnologies and increase the number of animals. Moreover, this investigation included in the process with n = 10 reproducibly viable multiparous females were used, estrous was synchronized and the transrectal ultrasonography was performed during 27 consecutive days every 24 h (8:00 h) with DP 10 Mindray®, equipped with 7.5 MHz linear transducers. Ninety percent of the sampled population (9/10) responded to the synchronization protocol, of which n = 3 females showed two follicular waves during their Estrous Cycle (EC), with a duration of 20 ± 0.6 days. Meanwhile, that n = 6 females presented three follicular waves during the 22±0.5 days of their EC. The Corpus Luteum (CL) stays a few days longer (19±0.96 days) in individuals of three waves, while in the two lasts until day (17±1). In conclusion, the cows of the Caqueteño creole cattle breed tend to present three follicular waves during EC, having an impact on the increase of the interovulatory interval.

Keywords: Caqueteño Creole Cattle Breed, Follicular Waves, Estrous Cycle

Introduction

The Estrous Cycle (EC) is a coordinated event of ovarian events that induce the female to go from a period of non-reproductive receptivity to receptivity with a period of 2-24 h during the late follicular phase. This stage is also known as estrous or heat that allows mating and subsequent pregnancy. It has been described that the interval, between two heats, has a durability that ranges between 18-24 days with an average of 21 days (Forde *et al.*, 2011), influenced by gonadotropic hormones FSH and LH, considered as the main regulators of folliculogenesis and the steroidogenesis. The pulse frequency of Luteotropic main Hormone (LH) is the determining factor for the final destination of a dominant follice, that is to say, ovulation.

In the middle of each cycle there are changes in the ovarian parenchyma, developing structures defined as Follicles (FL, which initiate asynchronous growth that ends with ovulation and subsequent formation of the Corpus Luteum CL), induced by hormonal stimulation from the axis hypothalamus-pituitary-gonadal (Forde et al., 2011). This event is known as follicular dynamics, its conformation is based on the development of two or three follicular waves. Each wave is composed of the common growth phase which covers the recruitment and selection stage, that is, it extends from the beginning of the emergency to the beginning of the deviation (Ginther et al., 2003). During this period a cohort of follicles is developed, being nothing more than an antral follicular group (1-4 mm in diameter) that indicate the starting of a wave between day (d) 2 and 3 of the cycle (Peter et al., 2009). These follicles undertake harmonic and simultaneous growth during several days where either of them can become a Dominant Follicle (DF) once they have reached a diameter of 5 mm (Gibbons et al., 1997) (Ginther, 2016). It has been defined that there is another phase known as dominance, being the stage in which a single antral follicle is observed that usually reaches size \geq 10 mm and include codominant or subordinate follicles



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These events can be visualized when implementingtransrectal ultrasonography, considered a highly repeatable, non-invasive, innocuous and safe method that allows to evaluate the reproductive tract of the female, observing in detail the uterus and the ovaries, recognizing in them the functional structures (FL's& CL) which are easily distinguishable from the ovarian stroma (Corredor-Camargo and Páez-Barón, 2012).

The implementation of reproductive biotechnologies is aimed at improving the genetic characteristics of animals destined for production, in search of better performance in the face of climate change, feed conversion and resistance to the environmental conditions in which they are found. Moreover, being necessary the previous study of ovarian physiology to effectively manipulate the reproductive phenomenon, in order to implement programs such as Fixed-Time Artificial Insemination (FTAI), superovulation, ultrasound-guided follicular puncture and/or Embryo Transfer (ET) (Choudhary *et al.*, 2016).

Caqueteño creole cattle breedis classified as one of the eight Colombian creole cattle breeds, considered as a Bos Taurus genetic group (Barrera *et al.*, 2006). Its adaptation process has been extended for more than 500 years after the arrival to the American continent of the Spaniards, arising as a result of the mating between the different European breeds common in that epoch of conquest. These cattle were reproduced indiscriminately, allowing them to adapt to the environment of the different regions of Colombia (Hernández, 1981).

One of the aspects to highlight of the creole cattle breeds is their high genetic value, considered as irreplaceable due to the degree of adaptation to the climatic change and low-quality feeds, making animals of creole cattle breeds more productive under tropical and hot weather conditions than specialized European cattle breeds (Haro, 2003; Sastre *et al.*, 2010).

Nowadays, there are less than 350 specimens of the Caqueteñocreole cattle breed, cataloged as endangered breed (Gutierrez et al., 2003), being necessary the implementation of public policies that help to preserve and foment this zoogenetic resource in the country (Martínez Correal and Correal, 2010). In that way, it is important to establish programs for the conservation, multiplication, creation of breed germplasm banks and the use of reproductive biotechnologies. The biotechnologies are very desirable alternative when thinking about conservation, that is why it is necessary to know the reproductive physiology of the breed, contribution that aims to make the present study that it will help clarify the follicular dynamics, wave number and luteal behavior during a CE; since there are no

previous documents describing the ovarian follicular dynamics of Caqueteño creole bovine females.

Materials and Methods

The study was carried out at the CimazMacagual Amazonian Research Center "Cesar Augusto Estrada Gonzales", in the south of Colombia department of Caquetá, 20 km from its capital Florencia, geographically located at 37°N and 75° 36'W, at an altitude of 300 m above sea level, the research center is characterized by a tropical humid forest weather, average temperature of 25.5°C and precipitation of 3,793 mm per year (Estrada and Rosas, 2007).

The research was conducted with 10 multiparous cows of the Caqueteño creole cattle breed, older than 48 months of age, (40% of the cows had lactations greater than 45 days). The selection criteria were determined by: body condition, general clinical examination, compliance with the health plan and absence of diseases of official control.

The determination of the physiological state of the reproductive system was made by rectal examination supported with ultrasonography, the management of the selected animals did not imply changes in the usual feeding system. The feedind system was characterized by grazing in prairies of Brachiariadecumbens, B. humidicola, native pastures of the region, wate and mineralized salt ad libitum.

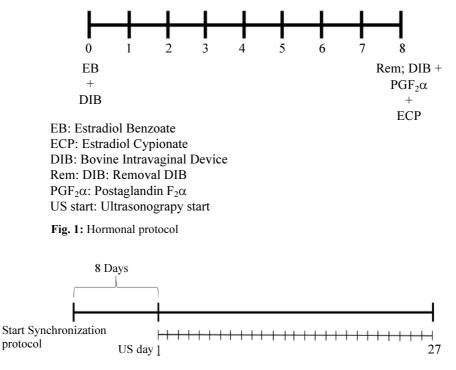
Protocol

The selected animals received hormonal treatment in order to synchronize the estrous cycle, carrying out synchronization protocol with estradiol salt (estradiol cypionate) prior to follicular dynamics monitoring (Fig. 1).

The protocol was executed in the following way: (day 0) application of intravaginal device impregnated with progesterone with a concentration of 0.5 g (DIB syntex®) and application of 2 mg of (estradiol® benzoate) via IM at day (8), the DIB was removed and 2mg of prostaglandin F2 α (cloprostenol) IM was applied, followed by the application of 2 mg of Estradiol Cypionate (ECP) IM on the same day (8) (Fig. 1). In addition, when the DIB was removed ultrasonographic evaluation began, as the day (1) of ultrasound.

Ultrasonographic Monitoring

To perform ovarian activity evaluation, a DP 10 Mindray® ultrasound scanner equipped with a 7.5 MHz linear transrectal transducer was used every 24 h (8:00 h) for 27 consecutive days (Fig. 2), registering the follicles that had size average \geq 3 mm in diameter, taken from two perpendicular measurements. The follicles were grouped according to their diameter in Class 1 [C1]: \leq 5 mm; Class 2 [C2]: 6-9 mm and Class 3 [C3]: \geq 10 mm according to the methodology applied (Diaz *et al.*, 1998).



US day: Ultrasonography day

Fig. 2: Synchronization protocol and ultrasonography

Statistical Analysis

The bases of the data were elaborated in Excel format and analyzed by means of the InfoStat software, giving as results descriptive statistics, comparison of mean and graphs of a polynomial tendency to determine the number and behavior of follicular waves of bovine creole cattle breed.

For the characterization of follicular behavior, the following concepts were used:

The appearance of follicular waves and duration: The anovulatory waves were determined by the difference in days between the emergenceday of the Dominant Follicle (DF) of two successive waves (Diaz *et al.*, 1998). In the case of the ovulatory wave, the day of the emergence of the pre-ovulatory follicle was taken into account until the day of ovulation.

Day of the follicular cohort of each wave (emergency): Day of the cycle where a group of antral follicles with size \geq 3 mm was found (Peter *et al.*, 2009).

Maximum day of recruitment in the follicular wave: It was determined when considering progressive increment in the size of follicles C1 (≤ 5 mm), accompanied by an increase in the number offollicles C2 (6-9 mm) in the same day (D'Enjoy *et al.*, 2012). Beginning of the selection of DF: Day of the wave in which there was an increase in the number of follicles C3 (\geq 10 mm) and a decrease in the number of follicles C2 (6-9 mm) (Diaz *et al.*, 1998). However, in the case of waves that did not present follicles greater than 10 mm in diameter, it was considered from the day after the maximum recruitment until the day before the deviation.

Beginning of the follicular deviation: Moment in which the DF (F1) was appreciated with size> 7 mm in diameter and there were significant differences in the growth rate between the F1 major follicle and the second F2 major follicle (Ginther, 2016; Ginther *et al.*, 2016).

Follicular dominance: Stage in which the largest follicle was observed once the deviation occurred (Peter *et al.*, 2009).

Ovulation: Disappearance of a Dominant Follicle (DF) identified in the previous US (Dorneles Tortorella *et al.*, 2016).

Results

Ninety percent (9/10) of the individuals evaluated responded to the synchronization protocol and presented normal cyclicity; in total n = 3 multiparous females with lactations> 45 days, showed two follicular waves during EC, ovulation occurred on day (d) 4.6 ± 0.68 of the US; while n = 6 multiparous females exhibited three follicular waves, these ovulated in d 4 ± 0.89 of the US, from this moment on it was indicated as d 0 of the EC.

Follicular Waves and Duration

A cohort of follicles measuring 4 ± 0.6 mm in diameter was observed during the day (d) 3 of the estrous cycle, which progressively increased in size, indicating the start of the 1st wave (anovulatory), which lasted 8 days. Likewise, during d 11th of EC, similar behavior was observed with an average follicular size of 4,1±0,9 mm, deducting the start of the 2nd wave (ovulatory), with a duration of 9 days, ending in the d 20±0,6 of EC, this being the day of ovulation.

In the case of cows that registered 3 waves (n = 6), a cohort of FL of $3,5\pm0,8$ mm in diameter was observed in the de 4 of EC, which presented a constant increase in their size, implying that the 1st wave (anovulatory) has begun. This same behavior was observed on days 10 and 17 of the EC, with a diameter of $4,3\pm0,8$ and $4,3\pm0,5$ mm respectively, inferring that in each case the 2nd (anovulatory) and the 3rd (ovulatory) follicular wave, with a duration of 6, 7 and 5 days, ending the EC at d 22 ± 0.5 with ovulation.

Common Growth Phase

The common growth phase, between the beginning of the emergence of the waves until the beginning of the deviation was developed and the follicles changed their diameter, so they abandoned a class (C1, C2, C3) to enter the next class, this behavior allowed to particularize each stage of the cycle. Further, in cows with 2 follicular waves, a first event was found during d 4 of the EC, in which the C1 follicles grew successively and the number of C2 follicles increased, suggesting that the maximum recruitment phase has been reached in the first follicular wave. Likewise, in d 11, follicle size C1 increases again and simultaneously the amount of FL C2, extending until d 14 of the EC, reflecting maximum follicular recruitment for the 2 waves. Similarly, in cows of 3 waves, the recruitment stage occurred on days 4, 10 and 17.

At the moment of describing the selection in cows of 2 waves, it was not possible to categorize the follicles by C3, because there were no diameters ≥ 10 mm. So, it was determined from the d following the maximum recruitment of each wave to the previous d to the deviation. Likewise, the selection started on d 5 and 15 and extended until d 6 and 18 of each wave. On the other

hand, it was observed that from d 11 the follicle that had greater size tended to decrease its diameter, indicating that it has lost its dominance, allowing to deduce that the 1st wave has finished and started the 2nd. As for the cows of 3 waves, the follicles with a diameter ≥ 10 mm were appreciated, favoring the classification. Thus, during days 5, 12 and 18 of EC, there was a decrease in the number of FL C2 and an increase in C3, revealing the phase of selection of each wave. In addition, on days 11 and 17 there was a decrease in the amount of C3, understanding that the larger follicle reduced its diameter so that the start of the regression in the anovulatory waves (1st and 2nd) is deduced.

Follicular Deviation

As previously mentioned, during this stage, there are significant changes in the growth rate between the largest follicles F1 and F2, which one of them will reach dominance and the next will suffer atresia. Further, at the time of visualizing the follicular behavior by means of polynomial trend lines (Fig. 3), it was found in the Caqueteño creole cows that the deviation was presented before F1 reached 8.5 mm in diameter, the difference in the growth rate was 1.0 mm and 0.5 mm in each wave for cows of 2 waves and 1.8 mm for those of 3 follicular waves (Table 1).

Follicular Dominance and Ovulation

During the study, it was found that DF is smaller in cows of 2 waves in relation to those of 3 follicular waves (Table 1 and Fig. 5), ovulation occurred in d 20 ± 0.6 in cows of 2 waves and d 22 ± 0.5 in the 3 waves. Moreover, the anovulatory waves recorded follicles of smaller diameter in relation to the ovulatory in the individuals of 3 waves by EC, while the cows of 2 waves showed an ovulatory FL of smaller diameter with relation to the DF reported in the anovulatory wave (Table 1).

Regression of the Corpus Luteum

Once the removal of oocytes occurred during ovulation, a complex structure was developed in the ovary regulated by multiple factors; participating in the follicular behavior (Skarzynski *et al.*, 2008). The Corpus Luteum (CL) has been considered as one of the reasons why the EC extends a few days more in cows of 3 waves (22-23 days) compared to cows of 2 waves (19-20 days) (Adams *et al.*, 2008). This is due to the fact that the regression of the CL varies according to the number of waves, occurring in the 16 in the bull cows of 2 waves and d 19 in those of 3 waves (Peter *et al.*, 2009). In Caqueteño creole cattle, it was found that the CL started the regression from 17 ± 1 in individuals of 2 waves, whereas in the 3 waves it occurred on day $19\pm0,96$ (Fig. 4).

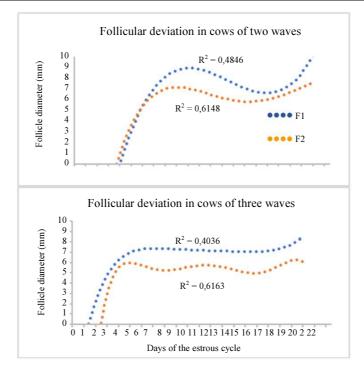


Fig. 3: Description of follicular behavior by means of polynomial trend lines during deviation

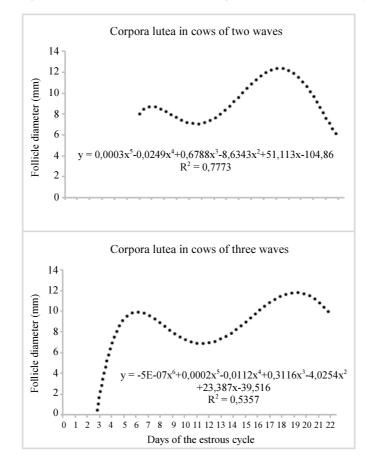


Fig. 4: Luteal behavior during the estrous cycle in Caqueteño creole cows

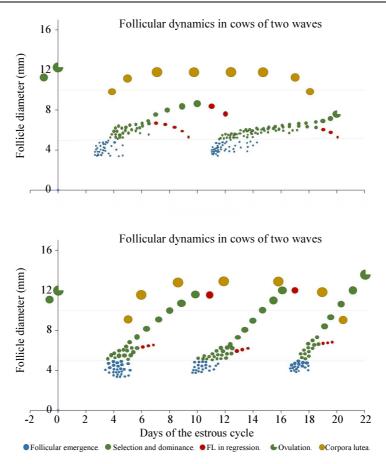


Fig. 5: Animation of follicular dynamics in multiparous Caqueteño creole cows

Discussion

There are numerous reports where follicular dynamics have been evaluated and characterized. These studies have provided sufficient information to establish parameters of ovarian behavior during EC. In general, bovine cattle have been reported to be more frequently composed of two or three follicular waves during a EC (>95%) (Adams *et al.*, 2008; Forde *et al.*, 2011; Ginther *et al.*, 2017).

In the present study, it was possible to determine by transrectal ultrasound monitoring that multiparous cows of Caqueteño creole cattle breed tend to present three follicular waves (66.6%), coinciding with investigations carried out on autochthonous breeds (Bostaurus); a study that included postpartum dairy cows, it was reported that 71% of the cows showed three waves during EC, while the rest of the cows showed four waves (Henao *et al.*, 2004). Another test executed in Ethiopia where they included F1 individuals (Boran B. indicus X Holstein B. taurus) reported that 44% exhibited three waves. However, no relation was found to a study carried out in Mexico which evaluated the ovarian behavior of a creole

bovine breed, demonstrating that 77.3% of the individuals sampled are of two waves and only 22.7% of three (Quezada-Casasola et al., 2014). The reason for the manifestation of two or three waves is still unknown, some reports in cattle Bostaurus suggest that the number of waves is variable, since the appearance of a third wave, it may be influenced by caloric stress and nutritional deficiencies (Adams et al., 2008; Price and Carrière, 2004). In effect, this hypothesis could explain the behavior presented by the Quezada-Casasola et al. (2014) in Mexico with a creole breed, where nutritional and environmental management was carried out in full lairage. On the other hand, other authors consider that the quantity of waves is influenced by the rate of follicular growth and the day when CL starts its regression (Ginther et al., 2003). Although, the reason for the behavior in the number of waves is not clear; it has been described that cows with three waves tend to have better fertility compared to two waves, justifying that the amplitude of each wave exerts effects on the vitality of the follicle, being beneficial for the third follicle that grows for fewer days, contrary to what happens in the individuals of two waves, who will have older follicles (Townson et al., 2002).

	Number of follicular waves in cycle	
	Two (n = 3) Mean \pm	Three $(n = 6)$ Mean \pm
First anovulatory wave		
Deviation		
Day of the estrous cycle	7	6
Diámeter F1 (mm)	7,5±0,15	7,6±1,2
Diámeter F2 (mm)	6,5±1,14	5,8±1,4
Difference in size (≠ mm)	1,0	1,8
Dominance		
Day of the estrous cycle	9,3±1,5	$9,8{\pm}1,7$
Maximum diameter (mm)	8,4±1,3	11,7±3,3
start of the regression (day)	11	11
Second anovulatory wave		
Deviation		
Day of the estrous cycle	-	13
Diámeter F1 (mm)	-	$7,2{\pm}0,8$
Diámeter F2 (mm)	-	$5,4{\pm}0,9$
Difference in size (\neq mm)	-	1,8
Dominance		
Day of the estrous cycle	-	$16,3\pm1,4$
Maximum diameter (mm)	-	12,2±4,4
start of the regression (day)	-	17
Ovulatory wave		
Deviation		
Day of the estrous cycle	19	19
Diámeter F1 (mm)	$7,1{\pm}1,0$	$7,9{\pm}1,1$
Diámeter F2 (mm)	$6,6{\pm}0,7$	$6,1\pm1,9$
Difference in size (≠ mm)	0,5	1,8
Dominance	,	,
Day of the estrous cycle	20 ± 0.6	$22\pm0,5$
maximum ovulatory diameter (mm)	$7,5\pm1,1$	13,8±3,6
Corpora lutea		· · ·
Diámeter (mm)	11,3±4,3	11,2±3,2
start of the regression (day)	17,0 = 1,0 17 ± 1	19±0.96

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It was found in the Caqueteño creole cows that the EC is of greater length when the cows manifest three follicular waves (22 ± 0.5 days), while in two waves, the EC is shorter (20 ± 0.6 days). Relating to results obtained n Holstein cattle; Sartori et al. (2004) confirmed the trend exhibited by individuals with three waves, the interovulatory interval being longer compared to females presenting two follicular waves (2 waves = $20,7\pm0,7$ days and 3 waves = $23,1\pm0,7$ days). Likewise, Chasombat et al. (2014) evaluated the ovarian dynamics in Bosindicus native heifers, finding differences in the length of the CE, being 19,44±0,13 days in heifers of two waves and 21,13±0,26 days in females of three follicular waves, both reports attributed the behavior of cycle lengthto the duration that the CL presented during a EC.

It has been established that the luteal phase is the longest stage of the interovulatory interval, comprising more than 80% of the estrous cycle once ovulation has occurred and until its regression (Peter *et al.*, 2009); from the moment in which CL decreases

in diameter or luteolysis exists, P4 levels decrease (Forde *et al.*, 2011), stimulating the growth, maturation and finalization of the ovulatory follicle (Adams *et al.*, 2008). However, if CL remains will induce the appearance of a new wave (Boer *et al.*, 2011). Also, this physiological event has been proved in Colombian Blanco Orejinegro (BON) creole cattle breed (Henao *et al.*, 2004). The results of the present investigation allow confirming these events, finding that the CL starts its regression on d 17 ± 1 in multiparous creole cows of two waves and 19 ± 0.96 days in individuals of three follicular waves (Fig. 4).

Deviation

After the major follicle (F1) has reached a considerable size, it induces the growth block of the next major follicle that accompanies it (F2), there being differences in the growth rate; reports have determined that the average difference between the two largest follicles starts with 0.5 mm in diameter (Ginther *et al.*, 2003). In cattle Bostaurus, it was

demonstrated that a follicle is prepared for deviation when present size greater than 7 mm and less than 8.5 mm in diameter (Ginther, 2016), agreeing with the results of the present investigation where it was observed that the deviation in all waves (anovulatory and ovulatory) occurred before 8.5 mm in diameter. Similarly, there was a relationship with studies in cows CurraleiroPé-Durobreed, adapted to tropical conditions, who did not surpass 8.5 mm during the deviation (F1: 7,9±0,2 and F2: 7,3±0,2). However, F1 and F2 diameters were not related (Dorneles Tortorella *et al.*, 2016), being lower in the Caqueteño breed (Table 1).

Dominance and Ovulation

It has been described that a dominant follicle is one that has reached the largest antral diameter after deviation (Peter et al., 2009). After dominance, the follicles belonging to ovulatory waves will come to term, while in the anovulatory waves they will suffer atresia because they remain under the influence of the corpus luteum which suppresses the growth of the follicles, preventing them from ovulation (Skarzynski et al., 2013). In general, reports show that follicles in Bostaurus cattle reach ovulatory capacity when they have a diameter >10 mm (Peter et al., 2009; Sartori et al., 2001), diverging with results from the present investigation, specifically in two-wave cows who presented ovulation at 7,5±1,1 mm (Table 1). No references were found in Bostaurus cattle that allow correlating the small ovulatory follicularsize found in individuals with two waves; while in Bosindicus cattle, it was found that some individuals acquired ovulatory capacity only 7 mm in diameter (Gimenes et al., 2008). Besides, Dorneles Tortorella et al. (2016) have reported that the diameter of the ovulatoryfollicle in Brazilian cows (CurraleiroPé-Duro) is lower than that reported in Bostaurus cattle.

Conclusion

Caqueteño creole cattle breed have two and three follicular waves with a greater tendency to three waves per EC, having an impact on the increase of the interovulatory interval. Also, it was observed that each wave is longer when individuals have two waves, the reason why there is the third wave can be attributed to the two events discussed in the present investigation (environmental, nutritional conditions and/or length of CL). Further, it is pertinent to indicate that future studies related to follicular dynamics in Caqueteño creole cattle breed could add the evaluation of hormonal behavior (P4, FSH, LH and E2) during CE, allowing corroborating or failing to rethink the hypotheses supported in this study.

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Author Contributions

All authors were actively involved in all stage of the work. The manuscript it has been read and approved by all authors.

Conflict of Interest

The authors have no personal financial or nonfinancial competing interest in the product.

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