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# OVULATION SYNCHRONIZATION IN eCG-TREATED GOATS AFTER A $PGF_{2\alpha}$-BASED SHORT TIME PROTOCOL DURING THE BREEDING SEASON

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## ABSTRACT

**The aim of this study was to evaluate whether the administration of 100 IU of equine chorionic gonadotropin (eCG) or human chorionic gonadotropin (hCG) at the time of the second injection of a double prostaglandin $F_2\alpha$ (prostaglandin $PGF_2\alpha$) protocol, applied 7 days apart, improves the synchronization of estrous activity and ovulation in goats. Twenty-four cyclic goats were assigned to three groups (n = 8 each). All animals received two doses of 5 mg $PGF_2\alpha$ (DIN), 7 days apart; at the time of second injection, they were treated with 100 IU of eCG (PG-eCG), 100 IU of hCG (PG-hCG), or no additional hormone (PG). Although the initial absolute estrous response at 36 h was similar between PG and PG-eCG groups, the PG-eCG treatment achieved a higher cumulative estrous response by 48 h and maintained this advantage at later timepoints (75 and 87.5%, respectively; $p<0.05$). Follicular monitoring revealed that, in does of the PG–eCG group, eCG administration promoted a more coordinated preovulatory follicular development, particularly between 36 and 72 h after the second DIN injection, and resulted in a concentrated ovulatory peak between 60 and 72 h. This pattern indicates superior synchronization compared with the broader ovulatory window observed in the PG group (36–96 h). In the hCG-treated group, the ovulatory response was distributed across a wide**

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**time window, from 36 to 84 h; furthermore, a null response to the treatment was also observed. There were no significant differences in estrous or ovulatory response between the PG and PG-eCG groups ($p > 0.05$); however, both showed higher responses compared with PG-hCG ($p < 0.05$). Goats treated with 100 IU of eCG after a synchronization treatment with a $PGF_{2\alpha}$ analogue showed a better estrous (shorter presentation time) and ovulatory responses. Therefore, it can be an alternative to make hormonal treatments more efficient at low cost. It can also be implemented in FTAI (fixed-time artificial insemination) protocols to positively impact on reproductive parameters.**

**Keywords:** synchronization, gonadotropins, short-term prostaglandins.

## INTRODUCTION

Reproductive efficiency in small ruminants can be enhanced through diverse management and nutritional strategies. However, when precise synchronization or fixed-time breeding is required, the control of the estrous cycle through exogenous hormones capable of modifying key physiological events of the sexual cycle remains essential (Abecia et al., 2012; Sun et al., 2024). Moreover, synchronization techniques have been adapted to promote sustainable practices by reducing the reliance on high doses of hormones (Gonzalez-Bulnes et al., 2020; Silva et al., 2022). It is therefore important to implement techniques that improve productive and reproductive parameters using treatments that are accessible to traditional producers and promote more efficient use of resources.

One of the treatments commonly used to synchronize estrous activity during the reproductive season is the administration of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) or its synthetic analogues (e.g., D-cloprostenol, DL-cloprostenol, delprostenate, or dinoprost) (Fierro et al., 2013; Olivera-Muzante et al., 2020; Santos-Jimenez et al., 2020). Dinoprost corresponds to the natural $PGF_{2\alpha}$, whereas cloprostenol is a synthetic analogue; however, the commercial formulations of both products used in veterinary medicine are manufactured synthetically. In goats, both compounds require a 2-day meat withdrawal period. As $PGF_{2\alpha}$ is rapidly metabolized in the lungs, its use contributes to animal welfare due to its intramuscular administration (Light et al., 1994). The mechanism of action of $PGF_{2\alpha}$-based treatments is to terminate the luteal phase by inducing lysis of the CL (Corpus Luteum) in cyclic females (Wildeus, 2000). Complete regression of the CL defined in the literature by the decline of circulating progesterone occurs within 6 to 24 h following administration (reviewed by Fierro et al., 2013). This leads to a new follicular wave that culminates in the onset of estrous activity and ovulation (Romano et al., 2017).

Initially, $PGF_{2\alpha}$ was administered as a single dose; however, low estrous responses were reported because not all females were cycling at the time of treatment. Therefore, its use became limited to females that were effectively in a luteal phase. In addition, the efficacy of $PGF_{2\alpha}$ depends on the functional state of the CL. It is generally accepted that the CL during its early development is not responsive to exogenous $PGF_{2\alpha}$, and luteolysis can only be induced once the CL reaches functional maturity (Rubianes et al., 2003). To overcome the limitations of single-dose protocols, two $PGF_{2\alpha}$ applications administered 9 to 13 days apart began to be evaluated, an interval that closely simulates the normal duration of the luteal phase of the estrous cycle (Ahmed et al., 1998; Fonseca et al., 2005). The benefit of this strategy lies in ensuring that all females regardless of whether they had a functional CL at the time of the first administration are in a luteal phase with a responsive CL at the second dose. Consequently, the proportion of females undergoing luteolysis and expressing estrus is markedly increased. Likewise, short 7-day protocols have been tested, yielding similar estrous responses within 25 to 48 h after the second injection (Menchaca et al., 2004). Although prostaglandin-based protocols are cost-effective for estrus synchronization, they are not suitable for fixed-time artificial insemination (FTAI) because the wide dispersion in ovulation compromises the precision required for fixed-time breeding (Olivera-Muzante et al., 2020).

Therefore, the efficacy of $PGF_{2\alpha}$-based synchronization combined with equine chorionic gonadotropin (eCG) has been evaluated in long-interval protocols (e.g., 14 days) to improve the synchrony of ovulation (Cueto et al., 2020). eCG possesses both follicle-stimulating hormone (FSH)- and luteinizing hormone (LH)-like activity, which promotes the recruitment of multiple follicles and supports the growth of a dominant follicle (Sharif et al., 2022). As a result, when eCG is administered at the end of a progesterone-based protocol, females typically develop preovulatory follicles that ovulate approximately 60 h later. However, follicular development and the timing of ovulation after eCG administration at the end of a short (7-day) $PGF_{2\alpha}$-based regimen

remain unknown. In contrast, human chorionic gonadotropin (hCG) acts primarily through LH receptors and does not stimulate early follicular recruitment. Its physiological effects are mostly ovulatory or luteotropic, which explains why hCG has been used to induce the formation of accessory corpora lutea around 7 days after mating or artificial insemination (Rodrigues et al., 2022). When administered at the end of intravaginal progesterone protocols, hCG has yielded variable results in goats and sheep (Fonseca et al., 2005; Esteves et al., 2013; Dias et al., 2020; Santos-Jimenez et al., 2021; Bruno-Galarra et al., 2021; Doğan et al., 2023; Sharif et al., 2023; Cox et al., 2024), and its effect within short prostaglandin-based synchronization protocols remains undetermined.

In this sense, we hypothesize that the use of a gonadotropin at the time of the second dose of PGF2$\alpha$ can improve the ovulation synchronization and, consequently, improve fertility outcomes in IATF programs. This study aimed to evaluate the effect of administering 100 IU of eCG or hCG at the time of the second PGF2$\alpha$ treatment on the synchronization of estrous activity and ovulation in adult goats during the breeding season.

## MATERIALS AND METHODS

### Ethics and animal care

All procedures followed were in accordance with national (NAM, 2010) and international (FASS, 2010) standards for the ethical care and protection of the animals used in the study. It was also reviewed and approved by the Collegiate Investigation Body of the Agrarian Autonomous Antonio Narro University (Mexico), with reference number UAAAN-UL/38111-425501002-2815.

### Study location and experimental groups

A preliminary study was conducted during the breeding season (November) on a commercial farm located in Torreón, Coahuila, Mexico (25°37'2 "N and longitude 103°23'7"W). Twenty-four mixed breed dairy goats (Alpine, Saanen x Criollo) of 15-40 months of age, with an average weight of 43.5 ± 0.2 kg and a body condition score of 2.3 (on a scale from 0 very thin to 5 obese) were used. The females used in this study were reared in a semi-extensive production system, grazing for seven h a day (10:00 to 17:00). A preliminary evaluation was performed to rule out females with any reproductive pathology or disease, which included a clinical examination, assessment of body condition, and transrectal palpation/ultrasound to confirm normal reproductive status. In addition, the females were cycling, which was confirmed through previous ultrasonographic examinations performed before the second $PGF_{2\alpha}$ administration. Functional corpora lutea were identified using color Doppler ultrasonography, assessing luteal blood flow as an indicator of active and functional luteal tissue, as described by Bartlewsk (2019). For this measurement, a linear transducer (7.5 MHz frequency) was used rectally (Echo 3, Chison Co., Jiangsu, China). All goats were synchronized using two doses of 5 mg of a $PGF_{2\alpha}$ analogue (Dinoprost Tromethamine; DIN, Lutalyse®, Zoetis, Mexico) administered 7 days apart. On the day of the second administration, animals were randomly assigned into three groups: Group PG-eCG (n = 8), which received 100 IU of eCG (GonActive® eCG, Virbac, Zapopan, Mexico); Group PG-hCG (n = 8), which received 100 IU of hCG (Chorulon®, MSD, Mexico City, Mexico); and Group PG (n = 8), which did not receive any gonadotropin treatment at the time of the second $PGF_{2\alpha}$ injection.

### Variables evaluated

#### *Estrous activity (occurrence, duration, and estrous response)*

The onset and duration of estrus were recorded from day 0 (application of the second dose of DIN), and every 12 h for 5 days. Four bucks with an average weight of 62±0.5 kg of proven fertility were used, and were introduced into the pen for 10 min, and the female that remained immobile during mating was marked as a female in estrus. The female was then removed from the pen and subjected to directed mating.

#### *Preovulatory follicle scanning and estrus-ovulation interval*

Females in all groups showing signs of estrus were also monitored every 12 h, from the onset of estrus until the disappearance of the preovulatory follicle ≥6 mm. For this measurement, a linear transducer (7.5 MHz frequency) was used rectally (Echo 3, Chison Co., Jiangsu, China). The procedure consisted of rectal insertion of the transducer at a 45° angle, previously lubricated with a water-based lubricant. When the preovulatory follicles disappeared, ovulation was confirmed. This measurement was used to determine the interval between estrus and ovulation (time of onset of estrus - time of preovulatory follicular disappearance.

#### *Ovulated females, number and size of corpus luteum*

The percentage of ovulated females was determined using the following formula: (females with a corpus luteum / total females per group × 100). In addition, the ovulation rate was assessed

based on the number of corpora lutea present 10 days after the second DIN administration, and the size of the corpora lutea for each female was also recorded (Echo 3, Chison Co., Jiangsu, China).

*Pregnant females and number of embryos*

Pregnancy and embryo number were assessed by transrectal ultrasound on day 35 after the second DIN dose. During the examination, anechoic structures within the uterus, indicative of embryo sacs, were identified and counted. Females displaying these characteristics were classified as pregnant (females with embryos / total females per group × 100).

**Statistical analysis**

The data were previously analyzed for normality using a Shapiro test. The response variables were evaluated using a generalized linear model considering groups and hours as factors. A completely randomized model was used. In contrast, proportional variables analyzed using a generalized linear model with a binomial distribution and logit link function, which is appropriate for modeling binary or proportion data and accounts for the bounded nature (0–1) and non-normal distribution of these outcomes. The results were reported as the mean ± SD (standard deviation), and a significance level of $p<0.05$ was considered. All procedures were performed using the R Studio 4.0.5 program (The R foundation for statistical computing, Boston, MA, USA). Equation 1 was used as the mathematical model for statistical analysis:

$$Yijkl = \mu + mi + sj + eijkl \quad (1)$$

Yijkl: observation value of the parameter
μ: overall mean of the parameter
mi: effect of reproductive response by group
sj: effect of reproductive response per hour
eijkl: random error variance.

## RESULTS

Fig. 1 shows that, 36 h after the second administration of DIN, a statistically significant response was observed in the PG and PG-eCG groups compared to the PG-hCG group (37% and 37% versus 11%, respectively; $p<0.05$). However, after 48 h, a higher accumulated response was observed in the PG-eCG group (75%), followed by the PG group (50%) and the PG-hCG group (33%; $p<0.05$). On the other hand, a similar accumulated estrous response was obtained in the PG and PG-eCG groups compared with the PG-hCG group (87.5, 87.5, and 44%, respectively; $p<0.05$).

Fig. 2a depicts the pattern of preovulatory follicle development (≥6 mm). The eCG-treated group exhibited a marked increase in follicular activity, particularly around 60 h after the second DIN application ($p<0.05$), accompanied by a more synchronized growth period between 36 and 72 h. In contrast, Fig. 2b shows that ovulations in the PG group occurred over a wider time span (36-96 h), whereas the PG-eCG group displayed a narrower and more defined ovulatory window (60-72 h), reflecting improved synchronization.

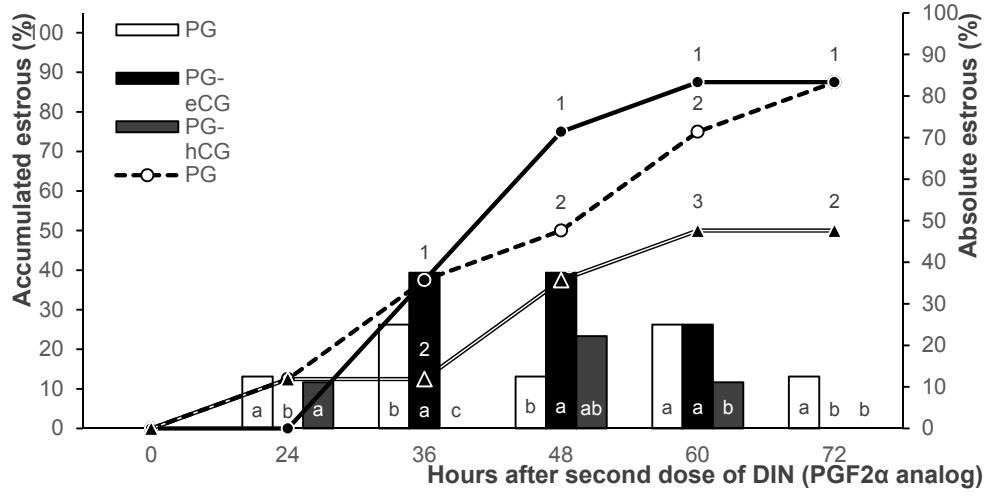


**Fig. 1. Cumulative percentage of estrous response (lines). [1, 2, 3] indicate statistical differences in lines ($p<0.05$). Absolute percentage of estrous response (bars). [a, b, c] indicate statistical differences among bars ($p<0.05$).**

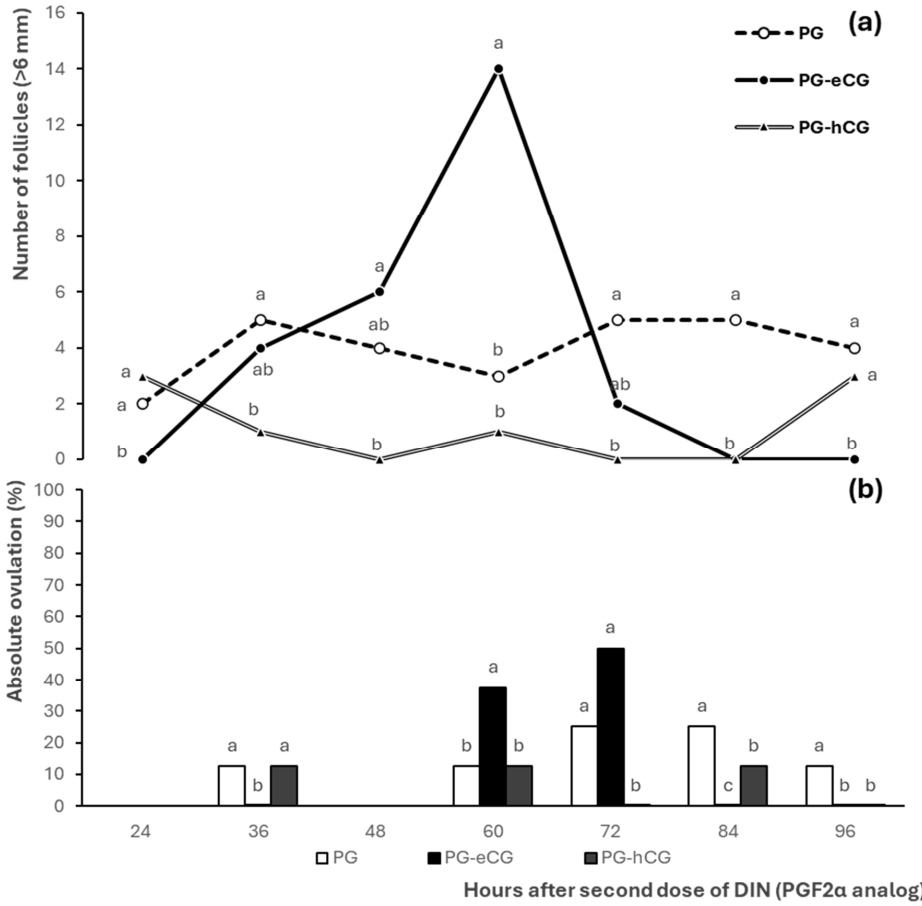


**Fig 2(a). Total follicles per group ≥6 mm. (b) absolute percentage of ovulations. a,b indicate statistical difference (p<0.05).**

Additionally, in the hCG-treated group, the ovulatory response was distributed across a wide time window, from 36 to 84 h; furthermore, a null response to the treatment was also observed.

The reproductive response is shown in Table 1. There were no significant differences between the PG *vs.* PG-eCG groups (p>0.05) in terms of estrous and ovulatory response. However, when comparing these two groups with respect to PG-hCG, they were different (p <0.05).

## DISCUSSION

The main objective of this research was to demonstrate whether the use of gonadotropins (eCG or hCG), based on the administration of two doses of a prostaglandin analogue at a 7-day interval, could be a more effective alternative for synchronizing estrus and ovulation and, consequently, determine the most appropriate time for insemination. It is well known that the manifestation of the estrous response in small ruminants, with the use of prostaglandins depends on the phase of the cycle and directly on the size of the preovulatory follicle present at the time of application of $PGF_{2\alpha}$. If the dominant follicle is smaller, it will take longer to mature and ovulate (Menchaca and Rubianes, 2004). On the other hand, if a larger follicle is present when $PGF_{2\alpha}$ is applied, it will reach the preovulatory phase faster and ovulate in less time (Fierro et al., 2013). For this reason, synchronization the estrous response with prostaglandins has not had the potential to be used as a tool in fixed-time insemination due to its low efficacy in synchronization ovulation.

In the present study, it was observed that goats that were not treated with gonadotropins (PG-group), a more prolonged time window of estrous activity was observed (Fig. 2a). This response was similar to that reported in other studies, where it is mentioned that estrous activity after two doses

**Table 1. Reproductive response of goats treated with PG, PG-eCG and PG-hCG.**

| Variables evaluated | Groups | | |
|---|---|---|---|
| | PG | PG-eCG | PG-hCG |
| Occurrence of estrus (h) | 49.7 ± 6.6 | 44.57 ± 3.2 | 48 ± 8.5 |
| Estrous response (%) | 87.5 (7/8)a | 87.5 (7/8)a | 50.0 (4/8)b |
| Estrous duration (h) | 39.4 ± 3.2 | 32.6 ± 4.0 | 36 ± 4.2 |
| Interval estrus/ovulation (h) | 34.3 ± 2.9 | 36.0 ± 2.6 | 27.0 ± 0.2 |
| Occurrence of ovulation (h) | 84 ± 6.9 | 78.0 ± 2.3 | 75 ± 6.8 |
| Ovulation day 10 (%) | 87.5 (7/8)a | 87.5 (7/8)a | 50.0 (4/8)b |
| Ovulation rate | 1.5 ± 0.1 | 1.7 ± 0.1 | 1.5 ± 0.1 |
| Size of the corpus luteum (mm) | 11.8 ± 0.1 | 11.9 ± 0.7 | 13.3 ± 0.2 |
| Pregnancy 35 days (%) | 75 (6/8) | 75 (6/8) | 50.0 (4/8) |
| Embryo rate (%) | 1.5±0.1 | 1.7±0.1 | 1.8±0.1 |

[a,b,] indicate differences between groups ($p<0.05$).

of $PGF_{2\alpha}$ treatment can occur from 24 to 96 h after the second administration at intervals of 10, 12, 14, 15 or 16 days (Fierro et al., 2016; Burutaran et al., 2023). For this reason, when used in IATF programs, more than one insemination should be performed (Olivera-Muzante et al., 2020).

On the other hand, the results obtained in the group treated with 100 IU of eCG at the time of the second administration of $PGF_{2\alpha}$ are promising, as the synchronization of the number of preovulatory follicles (≥ 6 mm) can be observed. A better synchronization of follicular growth is observed, which is related to the recruitment of a greater number of antral follicles in the follicular wave present. Therefore, the estrous response is synchronized more efficiently (Martinez-Ros and Gonzalez-Bulnes 2019). In the present study, it was observed that almost 80% of estrous response was observed in females treated with eCG (PG-eCG) after 48 h, ending with a 87.5% response at 60 h. In addition, the PG-eCG group showed a higher number of pre-ovulatory follicles (≥ 6mm) at 60h, indicating a synchronized follicular response between 36-60 h, creating a 24 h estrous window with positive implications to be implemented in FTAI protocols. Before, regarding the total number of follicles in the eCG group at 60 h (14 follicles), it was demonstrated that a low dose of 100 IU can stimulate the growth of a greater number of follicles with ovulatory capacity. It has been widely reported that the use of eCG enhances the reproductive response by activating steroidogenesis, promoting ovarian follicular growth, and facilitating oocyte maturation and follicular development (Wildeus, 2000).

There is evidence that, during the breeding season, the implementation of strategies to synchronize estrus during a limited period (24-72 h) can improve the use of artificial insemination (AI) or direct mating, with the objective of concentrating labor costs (Yu et al., 2022). In addition, follicles with preovulatory size ≥5 mm show a positive correlation with estradiol levels (Letelier et al., 2011); in turn, estradiol induces estrous behavior and simultaneously acts through a positive feedback mechanism to increase gonadotropin-releasing hormone (GnRH) secretion, inducing the preovulatory LH peak, causing ovulation between 20-26 h after the onset of estrus (Fatet et al., 2011). Cueto et al. (2020) evaluated the application of 200 IU of eCG at the end of a 14-day interval double $PGF_{2\alpha}$ estrous synchronization protocol in ewes and reported higher fecundity rates for eCG-treated ewes compared to the $PGF_{2\alpha}$-only control group. These findings provide valuable insights into how the addition of a gonadotropin, such as eCG, can significantly contribute to establishing a narrower time window for the reproductive response, which may have important implications as a biotechnological tool. Taken together, and in agreement with Sharif et al. (2022), the efficacy of eCG depends largely on the reproductive status of the females. In the present study, all goats were cycling, which likely contributed to the positive synchronizing effect of the gonadotropin. Therefore, the results should not be interpreted as a universal alternative to progesterone-based protocols, particularly in non-cycling does.

With regard to the hCG group, a low efficacy (50%) in inducing estrous activity was observed. This could be due to the fact that this gonadotropin has a main LH effect, which rapidly affects the luteinization of preovulatory follicles (Parmar, 2015). Similarly, a second minimal effect of hCG was largely due to its action on follicles that

were already of ovulatory size, as the biological effect of LH is to stimulate ovulation and, consequently, inhibit estrous activity. A study conducted by Esteves et al. (2013) evaluated the effect of the administration of 250 IU of hCG at the time of estrous manifestation in goats synchronized with two doses of PGF2$\alpha$ at 10-day intervals and reported no positive effects on fertility rates. Although different researchers have postulated that hCG is a good alternative for use in estrus synchronization programs, given its pharmacological properties, the results obtained have been highly variable (Fonseca et al., 2005; Al Yacuoub et al., 2011). Rodrigues et al., (2021) found that the administration of hCG prior to synchronization treatment may have a limited effect on the development of the preovulatory follicle.

## CONCLUSIONS

Goats treated with 100 IU of equine chorionic gonadotropin (eCG) after a synchronization treatment with a prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) analogue showed a better estrous (shorter presentation time) and ovulatory responses. Therefore, it can be an alternative to make hormonal treatments more efficient at low cost. It can also be implemented in FTAI protocols to positively influence reproductive parameters. Nevertheless, additional research with expanded sample sizes is necessary to validate these outcomes and improve their generalizability.

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**Author Contributions**

The authors declare active participation in the bibliographic review: Juan Manuel Guillen-Muñoz, Osiris Ortiz-Contreras and Zurisaday Santos-Jimenez; in the development of the methodology: Osiris Ortiz-Contreras, Luis Antonio Luna-García, Juan Luis Morales-Cruz and Hugo Zuriel Guerrero-Gallego; in the discussion of the results: Juan Manuel Guillen-Muñoz, Nesrein M. Hashem, Fernando Sanchez-Davila and Zurisaday Santos-Jimenez; in review and approval of the final version of the article: Juan Manuel Guillen-Muñoz, Nesrein M. Hashem, Fernando Sanchez-Davila and Zurisaday Santos-Jimenez.

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