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# **Does anthelmintics at the beginning of the FTAI protocol improve the pregnancy rate in Nellore cows?** Anti-helmínticos no início do protocolo de IATF melhoram a taxa de prenhez em vacas Nelore?

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**Abstract**
The objective of this research was to evaluate the influence of Levamisole and Moxidectin at the beginning of the FTAI protocol on the pregnancy rate in Nellore cows. The influence of body condition score (BCS), animal category, inseminator efficiency, heat intensity, and breeder on fertility was also analyzed. On D0 of FTAI, the animals were distributed into 3 treatments (Control, Levamisole and Moxidectin), in which they received, respectively, 1 mL/40 kg of 0.9% saline solution; 4.7 mg/kg Levamisole phosphate or 200 µg/kg Moxidectin, all subcutaneously. On the same day (D0), feces were collected. The intensity of estrus was assessed on the day of FTAI (D11) by marking the females' rump. The BCS was assessed on D0, on the day of TAI (D11) and 30 days later (D30). Pregnancy diagnosis was determined on D30 by ultrasonography. The data were submitted to the chi-square test. The dewormers did not interfere with the pregnancy rate, which demonstrates that, in the absence of parasitism, they do not promote a beneficial effect on reproduction. No effect of animal category and properties was observed (P>0.05), however, there was a significant influence of ECC, inseminator, heat intensity and bull semen´s on the pregnancy rate (P<0.05). An average pregnancy rate of 43.03% was observed for ECC between 2.5 and 3.5 and a pregnancy rate of 55.94% for females with heat score 0. Therefore, the nutritional and hormonal condition of the female interferes with the reproductive potential. However, the effect of the association of anthelmintics and FTAI protocols should be evaluated in situations of parasitism.
**Keywords**: Conception. Estrus. Dewormer. Beef cattle. Reproduction.

**Resumo**
Objetivou-se avaliar a influência do Levamisol e Moxidectina no início da IATF sobre a taxa de prenhez em vacas Nelore. O efeito do escore de condição corporal (ECC), categoria animal, inseminador, intensidade do cio e reprodutor sobre a fertilidade também foi avaliado. No D0 da IATF, os animais foram distribuídos em 3 tratamentos (Controle, Levamisol e Moxidectina), nos quais receberam, respectivamente, 1 mL/40kg de solução salina 0,9%; 4,7 mg/kg fosfato de Levamisol ou 200 µg/kg de Moxidectina, todos por via subcutânea. Nesse mesmo dia (D0), foi realizada a coleta de fezes. A intensidade do cio foi avaliada no dia da IATF (D11) por meio da marcação da garupa das fêmeas. O ECC foi avaliado no D0, no dia da IATF (D11) e 30 dias após (D30). O diagnóstico da prenhez foi determinado no D30 por ultrassonografia. Os dados foram submetidos ao teste qui-quadrado. Os vermífugos não interferiram na taxa de prenhez o que demonstra que, na ausência de parasitismo, não promovem efeito benéfico à reprodução. Não foi constatado efeito da categoria animal e das propriedades (P>0,05), porém, houve influência significativa do ECC, inseminador, intensidade do cio e reprodutor sobre a taxa de prenhez (P<0,05). Foi constatada taxa média de 43,03% de prenhez para ECC entre 2,5 e 3,5 e taxa de 55,94% de prenhez para as fêmeas com escore 0 de cio. Portanto, a condição nutricional e hormonal da fêmea interfere no potencial reprodutivo. Contudo, o efeito da associação dos anti-helmínticos e protocolos de IATF deve ser avaliado em situações de parasitismo.
**Palavras-chave**: Concepção. Cio. Vermífugo. Gado de corte. Reprodução.

## Introduction

Brazil stands out as having the largest commercial bovine herd and the largest exporter of beef in the world (ABIEC, 2023). However, despite the economic importance of beef cattle farming for agribusiness, the majority of herds are raised in extensive systems in native pastures without sanitary or reproductive control, which makes it difficult to expand the activity. Helminth-related disease can induce high morbidity in domestic animals and humans leading notable economic consequences (GHOLAMI et al., 2023). In the context of climate change and depletion of natural resource, controlling of these worms is crucial to attend the increasing demand for protein (AGRAWAL et al., 2024). Therefore, the development and adoption of sustainable strategies that optimize productive and reproductive indices on properties are necessary (CARVALHO, 2017).

In this context, the use of broad-spectrum anthelmintics such as Levamisole and Moxidectin has been recommended as a potential tool for the sanitary control of animals. Its correct administration reduces economic losses and damages to beef cattle production systems. Levamisole is an important member of the imidazothiazole derivatives with little drug resistance (MAUGER et al., 2022). Besides reducing parasite load, this antiparasitic has immunostimulatory capacity as a secondary effect (CHANDY et al., 2016). This product acts on the immune system like thymopoietin hormone, which influences the maturation of T lymphocytes, stimulates the action of T cells and their response to antigens, enhances the production of interferons, increases the phagocytic activity of macrophages and neutrophils, stimulates cell-mediated cytotoxicity, and enhances lymphocyte production (SAJID et al., 2006; PRASAD et al., 2012). Due to its immunomodulatory properties, Levamisole has been utilized for treatment of various diseases such as rheumatoid arthritis, cancer and also viruses' infections (FIROZABAD et al., 2021).

Moxidectin, which belongs to the macrocyclic lactone family, has also been used successfully as anthelmintic. There are two subfamilies within the macrocyclic drug class, the avermectin including ivermectin and milbemycin including Moxidectin, whit structural and action differences (MWACALIMBA et al., 2024). Evidence demonstrate that Ivermectin can modulate innate immune response by suppressing pro-inflammatory cytokines, besides acting as a potent anti-parasitic drug (SADEQ et al., 2025). Moxidectin, on the other hand, acts by promoting direct neural inhibition of the parasite, but there are no reports yet of their immunostimulatory action (DELAYTE et al., 2006). Recent studies have shown the ability of this drug to promote improvements with satisfactory results in conception rates in beef cows parasitized subjected to time-fixed artificial insemination (HELLER et al., 2021).

However, the use of these anthelmintics associated with time-fixed artificial insemination protocols to increase the conception rate as result of strengthening the clinical and immunological condition, regardless of the degree of parasitism of the animals, is still controversial. So, in this study, the influence of administering the anthelmintics Levamisole and Moxidectin at the beginning of the FTAI protocol in Nellore females on the pregnancy rate was evaluated, considering the body condition score (BCS), estrus expression intensity, as well as the effects of the inseminator, cyclicality, and the bull.

## Material and methods

*Animals and experimental design*

The project was approved by the Ethics Committee on Animal Use (CEUA) from the Federal Institute of Northern Minas Gerais (IFNMG) and Federal University of Minas Gerais (UFMG) under protocol numbers 018/19 and 351/2019, respectively. The experiment took place during the breeding season at three distinct properties located in the northern region of Minas Gerais state.

On each farm, 180 Nellore beef females from each category (heifers, primiparous, and multiparous) were used, totaling 540 animals. All heifers were submitted to puberty induction protocol, which involved the insertion of a fourth-use progesterone device (CIDR) that remained for 12 days. Upon removal of the device, 0.6 mg of estradiol cypionate (ECP) was administered. This procedure was adopted to ensure that this animal category had a similar stage of cyclicality in all properties.

At the time of gynecological evaluation, females were randomly distributed among three experimental groups: Control, where 0.9% saline solution at a dosage of 1 mL/40kg was administered subcutaneously; Levamisole group, where the females received 4.7 mg/kg Levamisole phosphate subcutaneously, equivalent to 1 mL/40kg of body weight (BW); and Moxidectin group, where the animals received 200 µg/kg of Moxidectin subcutaneously, equivalent to 1 mL/50kg of BW. In all treatments, a single dose of anthelmintics (Levamisole and Moxidectin) and/or saline solution (Control) was administered on the same day (D0) as the insertion of the intravaginal progesterone device (P4).

All animals were subjected to the same hormonal protocol consisting of four stages, including the insertion of the intravaginal P4 device (1.9 g of P4; CIDR®; Zoetis, SP, Brazil) and application of 2 mg of estradiol benzoate intramuscular (IM) (BE; Gonadiol®; Zoetis, SP, Brazil) at the beginning of the protocol (D0). Dinoprost tromethamine at a dosage of 12.5 mg was administered via IM (Lutalyse®; Zoetis, SP, Brazil) on the seventh day of the protocol (D7). The removal of the progestagen device was performed on the ninth day of the protocol (D9), along with the administration of 0.6 mg of estradiol cypionate IM (ECP®; Zoetis, SP, Brazil), 300 international units (IU) of equine chorionic gonadotropin (eCG) IM (Novormon®; Zoetis, SP, Brazil), and 12.5 mg of dinoprost tromethamine IM (Lutalyse®; Zoetis, SP, Brazil). Finally, on the eleventh day (D11) of the protocol, timed artificial insemination (TAI) was performed.

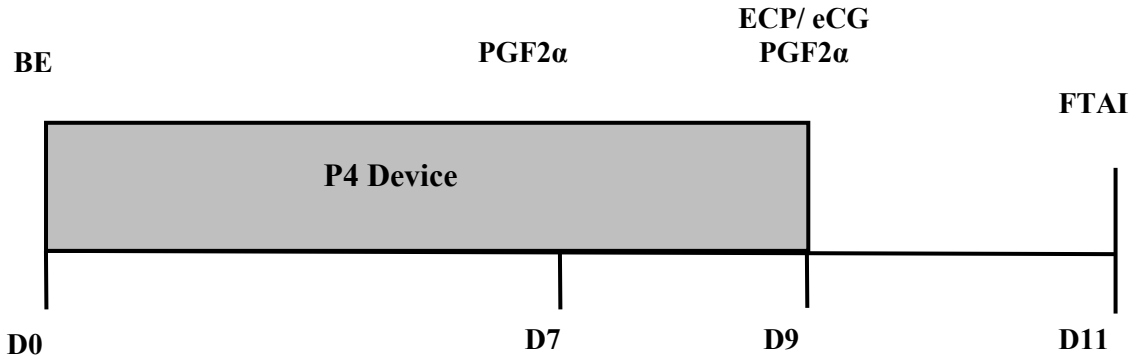


Figure 1 - Scheme of the FTAI hormonal protocol used in Nellore beef females. D0: insertion of intravaginal P4 device (1.9 g of P4; CIDR®; Zoetis, SP, Brazil) and application of 2 mg of estradiol benzoate intramuscular IM (BE; Gonadiol®; Zoetis, SP, Brazil). D7: 12.5 mg Dinoprost tromethamine IM (Lutalyse®; Zoetis, SP, Brazil). D9: Removal of the progestagen device and administration of 0.6 mg of estradiol cypionate IM (ECP®; Zoetis, SP, Brazil), 300 international units (IU) of equine chorionic gonadotropin (eCG) IM (Novormon®; Zoetis, SP, Brazil), and 12.5 mg of dinoprost tromethamine IM (Lutalyse®; Zoetis, SP, Brazil). D11: timed artificial insemination.

*Evaluation of BCS and FEC*

On the day of protocol initiation (D0), on the day of FTAI (D11), and after 30 days (D30), the body condition score (BCS) of the animals was evaluated using visual assessment on a scale of 1 to 5, according to Pfeifer et al. (2021). On D0, fecal samples were collected directly from the rectal ampulla of each cow for fecal egg count (FEC) examination, following the technique described by Gordon and Whitlock (1939).

*Estrus evaluation*

On the day of removal of the intravaginal progesterone device (D9), the tail base and rump of the animals were marked with paint sticks for identification of estrus intensity, according to the methodology described by Catalano et al. (2024).The estrus intensity was evaluated on FTAI Day (D11) and classified as 0 for animals with intense estrus without traces of paint; 1 for animals with moderate estrus, with some traces of paint on the rump; and 2 for animals that did not show estrus, with the paint remaining intact.

*Semen and inseminator*

Inseminations were performed with Nellore Bulls semen from insemination centers, tested and approved for FTAI (Brazilian College of Animal Reproduction 2013 - CBRA). More than two bulls were used per treatment. Artificial inseminations were performed by three different trained professionals on each farm.

*Pregnancy diagnosis*

Pregnancy diagnosis was performed 30 days after FTAI (DG30) using a portable ultrasound device (Mindray DP 2200) equipped with a linear transducer with a 5 Hz frequency. For this procedure, the animals were led to the restraining chute and handled with minimal stress.

*Statistical analysis*

The experimental design used was a randomized complete block design in a 3x3 factorial arrangement, with 3 experimental groups (Control, Levamisole, and Moxidectin), 3 animal categories (nulliparous, primiparous, and multiparous), and 3 blocks (each farm representing a block), with 60 animals per replication, totaling 1.620 animals. The chi-square test was used to evaluate the association between pregnancy rate and other parameters such as use of anthelmintics, animal category, farm, inseminator, estrus cyclicity, bull, estrus intensity, and body condition score. Analyses were conducted using SAS software (2014) with a significant level of 5%.

**Results**

The anthelmintics Levamisole or Moxidectin used for deworming the animals on D0 of the FTAI hormonal protocol showed no correlation with the pregnancy rate, as both treatments had

similar results, a mean of 42.08%, which was similar to the Control group. There was also no influence of the animal category on the pregnancy rate (Table 1, p = 0.1263, $\chi 2c = 4.138$). In the same way, the pregnancy rate did not vary significantly among properties, with values around 41.69% at Farm A, 49.43% at Farm B, and 46.14% at Farm C (p = 0.1146, $\chi 2c = 4.3325$).

Table 1 - Category influence on the efficiency of treatments in terms of pregnancy rate in Nellore beef females

| | **Multiparous (%)** | **Primiparous (%)** | **Nulliparous (%)** | **Mean (%)** |
|---|---|---|---|---|
| **Control** | 38.59 | 50.00 | 47.58 | 45.39 |
| **Cydectin** | 43.95 | 48.83 | 44.45 | 45.74 |
| **Ripercol** | 43.71 | 39.44 | 50.75 | 44.63 |
| **Mean (%)** | 42.08 | 46.09 | 47.59 | |

Chi-square independence test (p-value = 0.1263; $\chi 2c = 4.138$)

The mean body condition score (BCS) was 2.76 ± 0.31 with a coefficient of variation of 11.35%. There was the interference of BCS on pregnancy rates, with the highest rates obtained from females with scores ranging between 2.5 and 3.25 (p = 0.0049, $\chi 2c = 20.284$), as shown in Table 2. The effect of the inseminator on pregnancy rate (Table 3) was also observed ($\chi 2c = 20.752$, p < 0.0001). Among them, inseminator 3 had the highest average pregnancy rate (54.54%), while the overall average for all treatments was approximately 45% pregnancy rate.

Table 2 - Influence of body condition score on pregnancy rate in Nellore beef females

| | **2.25 (%)** | **2.50 (%)** | **2.75 (%)** | **3.00 (%)** | **3.25 (%)** | **3.50 (%)** | **3.75 (%)** |
|---|---|---|---|---|---|---|---|
| **Control** | 32.25 | 45.25 | 45.09 | 54.23 | 25.00 | 25.00 | 18.5 |
| **Cydectin** | 28.00 | 41.93 | 45.83 | 60.93 | 38.75 | 32.25 | 28.12 |
| **Ripercol** | 25.23 | 47.02 | 45.07 | 51.42 | 45.40 | 35.12 | 22.15 |
| **Mean (%)** | 28.49 | 44.74 | 45.33 | 55.52 | 40.15 | 30.79 | 22.92 |

Chi-square independence test (p-valor: 0.0049; $\chi 2c$:20.284)

Table 3 - Influence of the inseminator on pregnancy rate in Nellore beef females

| | **Inseminator 1 (%)** | **Inseminator 2 (%)** | **Inseminator 3 (%)** |
|---|---|---|---|
| **Control** | 38.58 | 38.18 | 59.65 |
| **Cydectin** | 43.34 | 39.13 | 53.84 |
| **Ripercol** | 46.48 | 38.34 | 50.41 |
| **Mean (%)** | 42.80 | 38.55 | 54.64 |

Chi-square independence test (p-valor<0.001; $\chi 2c$: 20.752)

Three bulls' semen was used for FTAI (Table 4), resulting in a significant difference in the pregnancy rate (p-value < 0.001, $\chi 2c = 21.943$). The animals inseminated with bull 3 semen had an average pregnancy rate of 52.73%, while the animals inseminated with 1 and 2 bull semen had an average of 38.19% and 36.48%, respectively. In relation to estrus intensity, animals that showed estrus score 0 had a higher correlation (Table 5, p-value < 0.001, $\chi 2c = 16.942$) with the pregnancy rate (55.94%) compared to animals that had estrus scores 1 and 2 (41.13% and 28.29%, respectively).

Table 4 - Bull influence on the efficiency of treatments in terms of pregnancy rate in Nellore beef females

| | Bull 1 (%) | Bull 2 (%) | Bull 3 (%) |
|---|---|---|---|
| **Control** | 36.08 | 32.43 | 57.02 |
| **Cydectin** | 40.50 | 29.41 | 51.18 |
| **Ripercol** | 38.00 | 47.62 | 50.00 |
| **Mean (%)** | 38.19a | 36.48a | 52.73b |

Chi-square independence test (p-valor< 0.001; $\chi$2c: 21.943)

Table 5 - Influence of heat intensity on treatment efficiency in terms of pregnancy rate Nellore beef females

| | 0 (%) | 1 (%) | 2 (%) |
|---|---|---|---|
| **Controle** | 61.59 | 40.90 | 33.34 |
| **Cydectin** | 54.19 | 52.50 | 20.00 |
| **Ripercol** | 52.05 | 30.00 | 33.34 |
| **Mean (%)** | 55.94 | 41.13 | 28.89 |

Chi-square independence test (p-valor<0.001; $\chi$2c: 16.942). 0 = animals with intense estrus; 1 = animals with moderate estrus, and 2 = animals that did not show estrus

## Discussion

There are numerous species of parasites with different mechanisms of action that compromise the animal's immune system, affecting productivity, facilitating the appearance of secondary pathologies, and leading to economic losses. Gastrointestinal nematodes cause diseases in beef cattle worldwide, resulting in an important reduction in productivity (VAN DER VOORT et al., 2013; CHARLIER et al., 2018; STRYDOM et al., 2023). So, the effective control of helminth infections in livestock is crucial to well-being of animals and production efficiency (AGRAWAL et al., 2004).

The losses from bovine parasitism are generally more severe in tropical and subtropical regions compared to temperate regions due to the combination of high temperatures and high rainfall (STRYDOM et al., 2023), which promote parasite survival and maintenance throughout the year. In the present study, no parasitism was found in the animals by EPG. This absence can be explained due to the time of year in which the study was conducted. The breeding season was conducted from December to March, starting after the foot-and-mouth disease vaccination campaign in November, when all animals on the property receive broad-spectrum dewormers and vaccines according to each property's sanitary calendar. Furthermore, the properties had adequate management with pasture rotation.

The similar pregnancy rate results among farms occurred due to the similar management of the properties, with the use of water troughs with piped water in the pastures, rotational grazing, and good animal welfare practices. Additionally, the farms adopt a sanitary calendar, where the animals undergo regular deworming every 4 months as defined by the veterinarian. This condition also contributed to the absence of influence of the dewormers on the pregnancy rate since the interruption of the parasites' spoliative action and the consequent improvement in the clinical and immunological condition probably would be related to better pregnancy rates.

In a recent study that evaluated 6,320 primiparous and multiparous Nellore females in 3 distinct experiments during two consecutive breeding seasons, pregnancy rates of 39.7% and 34.1% (first experiment), 55.1% and 48.1% (second experiment), and 56.4% and 52.8% (third experiment) were observed for the groups treated with 200 μg/Kg PV of Moxidectin and saline solution, respectively, demonstrating the efficacy of the drug on fertility (HELLER et al., 2021). Important information is that animals in this research were 90 to 120 days without any application of any anthelmintic and had a positive OPG with the presence of nematode eggs.

In contrast, in the present study, the application of anthelmintics was not effective due to the absence of gastrointestinal worm infestation in the animals. So, according to our results, the idea that deworms studied could cause clinical and reproductive benefits regardless of the degree of parasitism in the animals was not proven. The application of anthelmintics is suggested, therefore, when there is no possibility of performing a prior EPG examination, as it does not cause any decrease in FTAI conception rates. The choice of anthelmintic depends on the observed challenge.

Several factors can interfere with the animal's body condition, such as age, season at parturition, sex, breed, and animal category. According to Santos et al. (2009), BCS is a subjective classification of animals based on muscle mass and fat cover through visual and/or tactile analysis. This factor is directly controlled by nutrition, where cow body reserves change according to the physiological-nutritional requirements and forage supply, directly influencing the manifestation of the first postpartum estrus (SARTORI; MOLLO, 2007). In the present study, as expected, better pregnancy rates (ranging from 44.74% to 40.15%) were observed in females with scores ranging between 2.5 and 3.25, while extremes BCS (2.25 and 3.75) resulted in pregnancy rates less than 30%. Numerous studies demonstrate how low energy levels in the diet have negative effects on ovarian activity. Females with low BCS (less than 2.5) at parturition (WILTBANK et al., 1962), low BCS at FTAI (MENEGHETTI; VASCONCELOS, 2008; CARVALHO et al., 2014), and poor postpartum nutritional plan (WILTBANK et al., 2022; SAMADI et al., 2013) have a longer anestrous period and a lower probability of becoming pregnant.

Evidence demonstrates that inseminators have an important role in the success of the FTAI. So, the training, recycling, and welfare of inseminators in FTAI programs are necessary (HAMID, 2015; RUSSI et al., 2010; RUSSI et al., 2009). Similar results were found in research conducted by Carvalho et al. (2019), demonstrating the direct influence of inseminators on fertility rates, as observed in this work. According to Severo (2009), the proper manipulation of semen and the application of semen at the correct time and place can interfere with reproductive efficiency. However, that requires well-trained inseminators combined with the importance of evaluating semen to certify sperm with physical and morphological characteristics suitable for the moment of insemination to ensure fertilization.

In the present study, all the bulls used underwent sperm evaluation because they were bulls from semen collection centers, with their batches tested and approved for FTAI. Despite the possibility of individual differences in reproductive potential among bulls, it is important to consider that the variation in conception rates per bull may be influenced by other factors like the efficiency of inseminators and the intensity of estrus expression.

The transport of sperm through the female's reproductive tract is under the influence of estradiol predominance during the estrus (HAWK; TANABE, 1986). The dependence of sperm transport on the effect of estradiol is associated with changes in uterine pH, as there is considerable evidence that uterine pH regulates sperm motility and viability (LAVANYA et al., 2022). Larimore

et al. (2015) described in their study that cows showing estrus before FTAI had a higher number of accessory sperm and better embryo quality compared to cows that did not show estrus.

Another relevant effect of estrus expression is observed in the secretions of glycoproteins by the oviduct (BUHI, 2002) and the regulation of the uterine environment caused by endometrial secretions (NAKAMURA et al., 2005). In a recent study published by Pereira et al. (2020), they showed that 4,718 females with estrus score 0 had a conception rate of 54%, 1,660 females with estrus score 1 had a conception rate of 40%, and 246 females with estrus score 2 had a conception rate of 31%, corroborating the present data found in this study.

Cows showing estrus have increased expression of genes related to the maternal immune system and adhesion molecules. In addition, there is negative regulation of genes associated with prostaglandin synthesis and maintenance of the corpus luteum (DAVOODI et al., 2016). Therefore, the expression of estrus and the previous increase in estradiol levels at ovulation are directly related to the improvement of the uterine environment due to the elevation of endometrial secretions and better embryo nutrition and indirectly related to the progesterone response through the regulation of its receptors, increasing the conception rate, as observed in this study where the best conception rate was correlated with higher estrus expression.

## Conclusion

The nutritional and hormonal condition of the female, reflected by BCS and heat intensity, respectively, interferes with the reproductive potential. With regard to the sanitary condition, the dewormers Levamisole and Moxidectin applied at the beginning of hormonal protocols for FTAI did not interfere with the fertility of Nellore bovine females that are not parasitized. Further studies are necessary to evaluate the effects of the association of these anthelmintics and FTAI protocols in parasitized females, in different locations and at different times of the year.

## Interest conflicts

There was no conflict of interest among the authors.

## Authors' contribution

Everton Tadeu Negrão Pereira – project designing, field work, interpretation of results and article writing; Geovana Samara Andrade Aguiar, Moises de Aguiar Maia and Francisco Eduardo de Freitas Neto – research development, field work; Rodrigo Augusto Cortêz Passetti and Felipe Gomes da Silva – planning and statistical analysis; André Luiz Mendes Athayde – corrections and translating the article and Letícia Ferrari Crocomo – research coordination, project designing, writing and reviewing the article.

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