SEASONALLY ANESTRUS GOATS MATED BY SEXUALLY ACTIVE BUCKS CAN BECOME REFRACTORY TO THE PRESENCE OF BUCKS †

[LAS CABRAS EN ANESTRO ESTACIONAL EXPUESTAS A MACHOS CABRÍOS SEXUALMENTE ACTIVOS PUEDEN HACERSE REFRACTARIAS A LA PRESENCIA DE LOS MACHOS]

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SUMMARY

Background. Anestrous goats can cycle and become pregnant after the introduction of sexually active bucks, but some of them become anestrus probably because they have been fertilized and early embryonic death occurred or because they have not been fertilized and become refractory to the stimulation of bucks. **Objective.** Determine whether goats that were mated by bucks become anestrus probably because of an early embryonic death or refractoriness to buck stimulation. **Methodology.** Vasectomized (n = 2) and intact (n = 2) bucks were rendered sexually active by exposure to artificially long days (16 h of light per day) followed by natural variations of photoperiod. Bucks were introduced into two groups of anestrous goats (n = 15 each) and remained with them for 53 days. Estrous behavior, ovulation and pregnancy rate were determined. **Results.** All goats came into estrus, ovulated and were mated by bucks at least once during the study. The proportions of females that displayed estrus with or without ovulation did not differ between the two groups within 9 days after the introduction of bucks. However, the proportion of goats that came into estrus with or without ovulation was greater in goats mated by vasectomized bucks from 20 to 28 and from 50 to 53 days after introducing the bucks ($P < 0.05$). Finally, the proportion of goats that ovulated after the introduction of the bucks, but later returned to anestrus did not differ in goats mated by vasectomized or intact bucks (14% vs 33%; $P > 0.05$). **Implications.** These results indicate that in nonpregnant goats, refractoriness to the continuous presence of the same bucks may explain at least in part, the return to anestrus of most goats that stop cycling after an initial sexual response to the introduction of bucks. **Conclusion.** Nonpregnant goats mated by sexually active bucks become in anestrus, probably because they become refractory to the presence of the same males.

Key words: caprine; male effect; estrous behavior; ovulation; reproductive seasonality

RESUMEN

Antecedentes. Las cabras en anestro estacional pueden ciclar y quedar preñadas después de la introducción de machos sexualmente activos, pero algunas de ellas caen en anestro probablemente porque son fertilizadas y ocurre muerte embrionaria temprana o porque no son fertilizadas y se vuelven refractarias a la estimulación de los machos. **Objetivo.** Determinar si después de apareadas, las cabras caen en anestro probablemente porque ocurre muerte embrionaria temprana o porque se vuelven refractarias a la estimulación del macho. **Metodología.** Machos vasectomizados (n = 2) e intactos (n = 2) fueron activados sexualmente por exposición a días largos artificiales (16 h de luz por día), seguidos de las variaciones del fotoperiodo natural. Los machos se introdujeron en dos grupos de cabras en anestro estacional (n = 15 cada uno) y permanecieron con ellas por 53 días. Se determinaron el comportamiento estral, la ovulación y la tasa de preñez. **Resultados.** Todas las cabras entraron en celo, ovularon y

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fueron apareadas por los machos al menos una vez durante el estudio. Las proporciones de hembras que mostraron celo con o sin ovulación no difirieron entre los dos grupos dentro de los 9 días posteriores a la introducción de los machos. Sin embargo, la proporción de cabras que entraron en estro con o sin ovulación fue mayor en aquellas apareadas por machos vasectomizados de 20 a 28 y de 50 a 53 días después de la introducción de los machos ($P < 0.05$). Finalmente, la proporción de cabras que ovularon después de la introducción de los machos, pero luego regresaron al anestro, no difirió en cabras apareadas con machos vasectomizados o intactos (14% vs 33%; $P > 0.05$). **Implicaciones.** Estos resultados indican que, en cabras no gestantes, la refractariedad a la presencia continua de los mismos machos puede, en parte, explicar el retorno al anestro de la mayoría de las cabras que dejan de ciclar después de una respuesta sexual inicial a la introducción de los machos. **Conclusión.** Las hembras no gestantes apareadas por machos sexualmente activos, caen en anestro, probablemente, porque se vuelven refractarias a la presencia de los mismos machos.

Palabras clave: caprinos; efecto macho; comportamiento estral; ovulaciones; estacionalidad reproductiva

INTRODUCTION

Most goat breeds from subtropical latitudes have seasonal reproductive patterns (Restall, 1992; Giriboni *et al*., 2017). In the Northern Hemisphere, seasonal anestrus occurs from March to August (spring to mid-summer) in goats, and bucks decrease their reproductive status from January to June (Delgadillo *et al*., 1999; Duarte *et al*., 2008). As this seasonal pattern is mainly synchronized by photoperiodical changes (Delgadillo *et al*., 2004; Zarazaga *et al*., 2011), the sexual activity of males and females during sexual rest can be stimulated with artificial photoperiod treatments (Chemineau *et al*., 2007). However, the sexual activity of anestrous females can also be stimulated via sociosexual interactions, particularly the phenomenon called the "male effect" (see review Delgadillo *et al*., 2009). The introduction of a buck to a group of seasonally anovulatory goats stimulates the display of estrous behavior with or without ovulation, during the first days after joining them (Ott *et al*., 1980; Chemineau, 1983; Pellicer-Rubio *et al*., 2007). The intensity of the sexual behavior displayed by bucks can modify the percentage of anestrous goats that ovulate and might become pregnant. Indeed, while less than 10 % of goats ovulate after mating with bucks displaying weak sexual behavior, more than 90 % of goats ovulate after being stimulated by sexually active bucks previously exposed to long days followed by natural photoperiod conditions (Delgadillo *et al*., 2002, Bedos *et al*., 2012; Chasles *et al*., 2016).

The sexual response of goats stimulated by sexually active bucks is similar among different studies, including i) first ovulation occurring 2-5 days after the introduction of bucks, associated with estrus in a variable number of goats; ii) most goats display a short luteal phase, ovulating again 6-9 days after the initial introduction of the bucks, generally associated with estrus and followed by a luteal phase of normal length, so most goats can become pregnant (Muñoz *et al*., 2016; Araya *et al*., 2016); iii) most goats that do not get pregnant from this second ovulation become anestrus after the regression of the second corpus luteum, with very few females ovulating again and coming into estrus 23-30 days after the initial introduction of bucks (Delgadillo-Sánchez *et al*., 2003; Araya *et al*., 2016). To the best of our knowledge, there are no studies characterizing the factors that might influence the maintenance of the cyclicity or the return to anestrus of goats that ovulated after the introduction of the bucks. It is possible that goats are fertilized but after an early embryonic death, females return to anestrus (Spencer, 2013). Another possibility is that females do not continue cycling because they become refractory to the stimulation of bucks (Restall, 1992). Considering that sexually active bucks display intense sexual behavior for approximately 2 months, and that they can stimulate the sexual activity in most goats, the aim of this study was to determine if refractoriness also occurs in does mated by vasectomized bucks, and therefore, with no early embryonic death.

MATERIAL AND METHODS

Animals and general management

This study was conducted in the Laguna region in the state of Coahuila in northern subtropical Mexico (latitude 26°23' N, longitude 104°47' W), where the photoperiod varied from 13 h 41 min of light at the summer solstice to 10 h 19 min of light at the winter solstice during the study. Goats used in the study were from local crossbreeds previously described (Delgadillo *et al*., 1999; Duarte *et al*., 2008). Briefly, females isolated from males do not cycle from February to August, whereas bucks isolated from females have their sexual rest period from January to June (Delgadillo *et al*., 1999; Duarte *et al*., 2008). Bucks and does were offered 2 kg of alfalfa hay (18% CP) and 100 g of commercial concentrate (14% CP; 1.7 Mcal/kg) per animal/day, with free access to mineral blocks and water during the study.

Photoperiodic treatment of bucks

We used sexually experienced vasectomized (n = 2) and intact (n = 2) bucks that were 3-5 years old, weighing 67 ± 3 kg (mean ± SEM) at the beginning of the study. Bucks were treated with artificial

photoperiod to make them sexually active according to Delgadillo *et al*. (2002). Briefly, males were allocated in a shaded open pen under natural photoperiodic conditions and exposed to 2.5 months of artificially long days (16 h of light per day) from November 1st using artificial and natural light. Artificial light was provided from 06:00 to 08:00 and from 18:00 to 22:00. On January 16th, males were allocated again under natural photoperiodic conditions. This photoperiodic treatment stimulates the sexual activity of bucks for approximately two months starting in March (Delgadillo *et al*., 2002; Bedos *et al*., 2010; Delgadillo *et al*., 2022).

Sexual response of bucks to the photoperiodic treatment

One week before the introduction of the bucks into the groups of females, the sexual behavior displayed by bucks was determined individually by exposing them for 10 min to a female in anestrus. The sexual behavior was assessed by the number of nudging events, and as expected, all males responded to the photoperiodic treatment displaying equivalent sexual behavior (range: from 70 to 85 events). In addition, sperm production was determined in intact bucks. Ejaculates were collected twice using an artificial vagina, one ejaculate by day. The total number of spermatozoa per day (volume x sperm concentration per ejaculate) and progressive sperm motility were determined in undiluted samples after semen collection using a phase contrast microscope (Delgadillo *et al*., 1999). Bucks produced equivalent total number of spermatozoa per ejaculate (range: from 3.7 to 4.1 x 10^9) and sperm progressive motility (from 3.5 to 4.0; the scale varied from 1 to 5).

Female goats and the male effect

We used two groups of 15 multiparous, 3-4 years old, seasonally anovulatory goats. Each group of females remained in an open shaded pen under natural photoperiodic conditions. Before the introduction of bucks, the ovaries were scanned with transrectal ultrasonography on March 5, 12 and 19 to confirm the absence of a corpus luteum in all the does in the three occasions. One group of females (body weight: 33 ± 2 kg; body condition: 1.9 ± 0.5; range 1 to 4; 1 = very lean, 4 = fat; Walkden-Brown *et al*., 1977) was mated by intact sexually active bucks, whereas a second group (body weight: 33 ± 2 kg; body condition: 1.9 ± 0.1) was mated by vasectomized sexually active bucks. The contact between males and females began on March 26 (Day 0) and ended on 18 May. This 53-day period was decided to cover a theoretical duration of three cycles after the introduction of the bucks. Each group of females remained in contact with the same two bucks throughout the experiment, but only with one male at the same time to prevent fights, and thus, accidents between males. Therefore, we exchanged the bucks in their respective group daily, ensuring also that they were more stimulated due to temporal separation. We also prevented a possible effect of male "novelty" by placing the males in shaded open pens adjacent to those of the females after the initial introduction (Delgadillo *et al*., 2015). These bucks remained separated from females only by an openwork wood barrier that allowed visual, olfactory, and nose-to-nose contact between sexes.

Sexual and reproductive responses to the male effect

Estrous behavior was recorded twice daily by direct visual observation from 08:00 to 09:00 h and from 18:00 to 19:00 h during the entire experiment. A female was considered to be in estrus when she stood immobile while the buck mated her (Chemineau *et al*., 1992). Ovulation was confirmed by plasma progesterone concentration. For this purpose, blood samples were daily collected from day 0 to day 38 and then every three or four days until day 53 after the initial introduction of males. All blood samples were obtained from the jugular vein at 08:00 h in 5-mL tubes containing heparin. Plasma was obtained after centrifugation at 3500 x *g* for 30 min and stored at -20°C until the hormone concentrations were measured. Plasma progesterone concentrations were determined by the immunoenzymatic assay reported by Canépa *et al*. (2008). The sensitivity was 0.25 ng/mL. The intra- and inter-assay CV were 4 and 8%, respectively. It was considered that a goat ovulated if it had at least two consecutive blood samples with progesterone concentrations ≥ 1.0 ng/mL (Chemineau *et al*., 2006). The pregnancy rate of goats mated by intact bucks was determined by the presence of embryos using transrectal ultrasound 37 days after the initial introduction of bucks.

Statistical analyses

The proportion of goats that came into estrus and that ovulated were compared using the Fisher exact probability test. Statistical analyses were performed using System Statistics (2009).

RESULTS

Response of females to the male effect

All goats exposed to vasectomized or intact bucks displayed estrus and ovulated at least once during the study (Figure 1; Table 1). The proportions of females that displayed estrus with or without ovulation within the first 9 days after the introduction of the bucks did not differ among goats exposed to vasectomized or intact males ($P > 0.05$; Table 1). By contrast, the

proportion of goats that came into estrus with or without ovulation was greater in goats exposed to vasectomized bucks than in those exposed to intact bucks from 20 to 28 and from 50 to 53 days after the introduction of bucks ($P < 0.05$; Table 1).

From the non-pregnant goats, 33% returned to anestrus. This percentage did not differ from that of goats mated by vasectomized bucks (14%; $P > 0.05$). Overall, 60% of the goats became pregnant. In these goats, plasma progesterone concentrations remained at the level of pregnant goats until the end of the study. Goats joined with the vasectomized bucks that did not return to anestrus remained cyclic until the end of study (Table 1). Individual profiles of plasma progesterone concentrations of goats joined with vasectomized or intact bucks are shown in Figure 2.

Table 1. Estrus and ovulatory responses of female goats that mated with intact or vasectomized sexually active bucks during seasonal anestrus.

	Goats displaying								
	Estrus with ovulation	Ovulation without estrus	Estrus with ovulation	Estrus with ovulation	Estrus without ovulation	Estrus with ovulation	Ovulation without estrus	Estrus	Estrus with ovulation
Goats exposed to	(0-4 days; %)		(5-9 days; %)	(20-28 days; %)		(38 – 40 days; %)		(51-53 days; %)	
Intact bucks	87 [a]	13 [a]	80 [a]	7 [a]	7 [a]	0 [a]	0 [a]	0 [a]	0
Vasectomized bucks	93 [a]	7 [a]	67 [a]	100 [b]	0 [a]	14 [a]	14 [a]	40 [b]	NA

[a,b] Values with different letters within each column are different ($P < 0.05$).
NA: Non available.

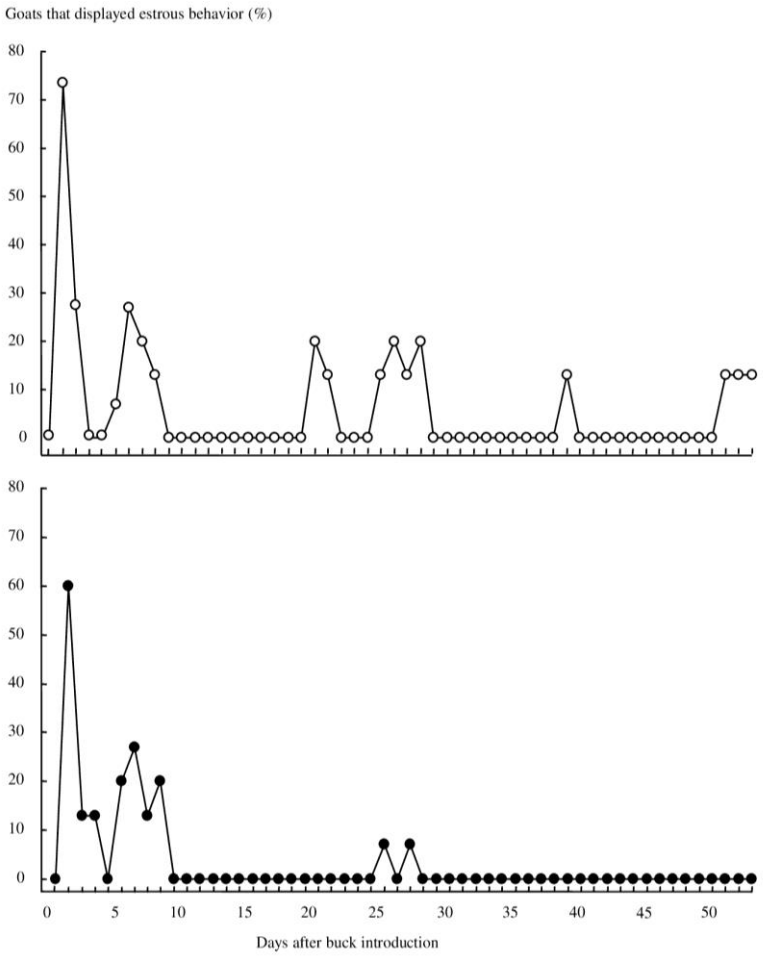

Figure 1. Percetages of goats that displayed estrous behavior after being stimulated by vasectomizaed (○) or intact (●) sexually active bucks during seasonal anestrus.

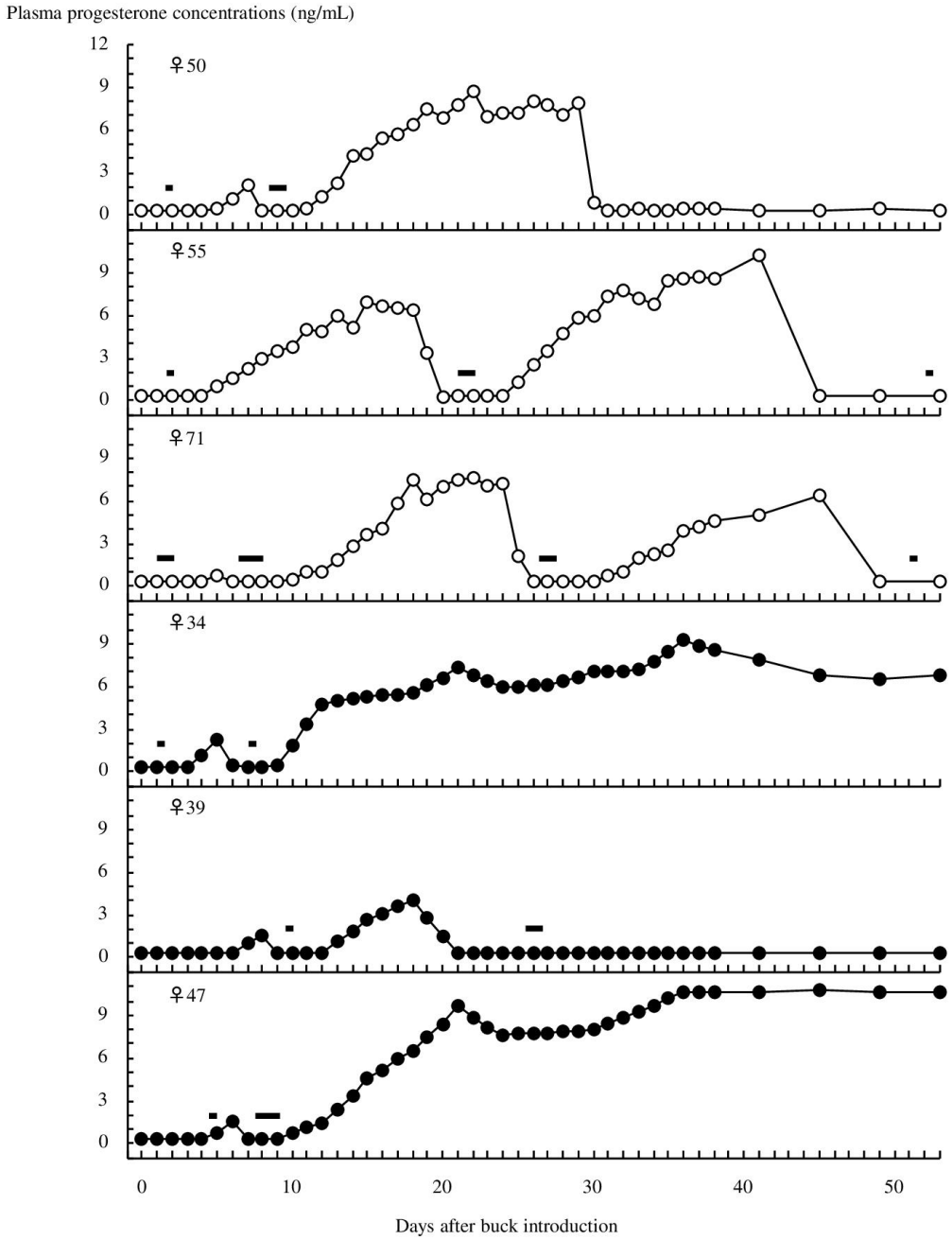

Figure 2. Individual profiles of plasma progesterone concentrations and estrous behavior representative of goats that were mated by vasectomized (○) or intact (●) sexually active bucks during seasonal anestrus. Estrous behavior that lasted from 12 to 36 h is indicated by a bold horizontal lines.

DISCUSSION

A similar proportion of goats returned to anestrus after being mated by sexually active vasectomized or intact bucks. With the experimental design used in the present study, is not possible to know if the goats that were mated by intact bucks were fertilized and their embryos died or oocytes were not fertilized. Therefore, it is not possible to discard the possibility of an early embryo mortality before the maternal pregnancy recognition. Thus, although not fully demonstrated, probably these goats also became refractory to the sustained stimulation of the bucks, as did females joined with vasectomized bucks. The existence of refractoriness to the stimulus of the active bucks is interesting, as it appears contradictory with previous studies in which female goats (Muñoz *et al*., 2016) and ewes (Jorre de St Jorre *et al*., 2012) respond to the introduction of males even after being in contact with other males. Moreover, Delgadillo *et*

al. (2015) and Abecia *et al*. (2015) demonstrated that both, goats and ewes, ovulate all the year-round if the males are exchanged. Therefore, it appears that the refractoriness is related to the sustained presence of the same males, and not to the previous contact with males per se, although this is probably also related to the anestrous individual physiological status.

As demonstrated in this study, while an important percentage of females continue cycling, others return to anestrus even when still being stimulated by the same bucks. Therefore, it appears that there is a thin thread determining individual females' responses to teasing. It is also interesting that apparently, although active males were able to trigger the endocrine changes that lead to ovulation, even being sexually active, their presence could not maintain cyclic activity. This coincides with previous results indicating that nonpregnant goats that were mated became anestrus even while remaining in contact with males displaying intense sexual behavior (Delgadillo-Sánchez *et al*., 2003; Araya *et al*., 2016). Our findings for goats that were mated by vasectomized bucks agree with those of Delgadillo *et al*. (2015) showing that 14% of goats in permanent contact with different vasectomized photoperiodic-treated bucks displaying intense sexual behavior from January to June, stopped cycling during seasonal anestrus. Therefore, our results, in conjunction with those of Delgadillo *et al*. (2015) indicate that the sexual activity of males is not enough to avoid the return to anestrus of goats that initially ovulated, reinforcing the explanation of females becoming refractory to the stimulus provided by bucks despite its' intensiveness.

The current study indicates that the initial reproductive response of goats to the introduction of bucks is related to the degree of stimulation of males, and not to the type of bucks used, i.e., vasectomized or intact. All goats exposed to vasectomized bucks displayed estrus associated with ovulation between 20 and 28 days after introducing the bucks, coinciding with results from other studies (Ott *et al*., 1980; Chemineau, 1983). The absence of a peak of estrous behavior on days 20 and 21 in goats that mated with intact bucks was probably due to the high fertility rate of goats that came into estrus and ovulated after the initial stimulation and produced a corpus luteum of normal length in response to the male effect. On the other hand, most goats exposed to vasectomized bucks also displayed estrus and/or ovulations until the end of the study, demonstrating that most goats can maintain their sexual activity for approximately two months, coinciding with the intense sexual behavior displayed by males rendered sexually active by exposure to artificial long days (Delgadillo *et al*., 2022).

CONCLUSIONS

These findings indicate that in seasonally anestrous-nonpregnant goats, refractoriness to the continuous presence to the same bucks may explain the return to anestrus of most goats that stop cycling after an initial sexual response to the introduction of the bucks.

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Data availability. The data that support the findings of this study are available from the corresponding author (joaldesa@yahoo.com) upon reasonable request.

Author contribution statement (CRediT). R.M. Aroña and A.L. Muñoz- Conceptualization, Investigation and Data curation; **H. Hernández-** Conceptualization and Formal analysis; **R. Ungerfeld-** Conceptualization and Methodology; **M. Keller and P. Chemineau-** Conceptualization and Methodology; **J.A. Delgadillo-**Conceptualization, Supervision, Resources and Project administration; all authors participated in the Writing - original draft and Writing – review & editing.

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