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Effects of daily propylene glycol drenching during the Ovsynch protocol on fertility and metabolic parameters in lactating dairy cows

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Abstract

Negative energy balance (NEB) caused by restricted feed intake leads to body condition loss (BCS), increased metabolic stress and reduced fertility in dairy cows. Propylene glycol (PG) is a precursor of ruminal propionate for gluconeogenesis used to increase metabolic adaptation to the early postpartum period. The aim of this study was to determine the effects of daily drenching of PG during the fixed-time artificial insemination (FTAI) protocol on beta-hydroxybutyric acid (BHBA), glucose, adiponectin, insulin-like growth factor-1 (IGF1) concentrations, follicle size and pregnancy rate in dairy cows. Cows ($n=148$) were randomly divided into two groups and received either 300 mL of PG (PG-OVS, $n=76$) or 300 mL of water (CON-OVS, $n=72$) each day of the Ovsynch protocol (GnRH-7 days-PGF$_{2\alpha}$-56 hours-GnRH-16-18-hour FTAI) between days 57 ± 3 to 67 ± 3 postpartum for the first service. Body condition scores (14 days before expected calving, at calving, on days 21 and 42 postpartum) were recorded. Blood samples were collected days 7 ± 3, 21 ± 3 postpartum, at the beginning of the Ovsynch (days 57 ± 3) and at the time of FTAI (days 67 ± 3) for measurements of BHBA, glucose, adiponectin and IGF1 concentrations. Ultrasonographic examinations were done to measure follicle size at the beginning of Ovsynch and FTAI and to determine pregnancy on days 30 and 60 following FTAI. There were no differences ($p>.05$) in glucose, adiponectin and IGF1 concentrations between the groups during the study. Although there was no difference ($p>.05$) in BHBA concentrations on postpartum day 7 ± 3, 21 ± 3 and 57 ± 3 between the groups, BHBA concentrations at the time of insemination was lower ($p<.05$) in the PG-OVS group (0.72 ± 0.03 mmol/L) than in the CON-OVS group (0.81 ± 0.03 mmol/L). Follicle sizes at the beginning of Ovsynch (PG-OVS, 14.5 ± 0.48 mm; CON-OVS, 14.3 ± 0.59 mm) and FTAI (PG-OVS, 17.8 ± 0.52 mm; CON-OVS, 17.7 ± 0.42 mm) were not different ($p<.05$). Pregnancy rate of the cows in the PG-OVS group (46.1%, 35/76) was higher ($p=.05$) than in the CON-OVS group (30.6%, 22/72) on day 30 following FTAI. In conclusion, decreasing serum BHBA concentrations at the time of FTAI by means of daily drenching of PG during the Ovsynch protocol, increased the pregnancy rate at first service in lactating dairy cows. On the other hand, blood glucose was not related with pregnancy rates in our study, probably as a result of our sampling time and more rapid fluctuations of blood glucose concentrations when compared to BHBA.

KEYWORDS
BHBA, dairy cow, fertility, Ovsynch, propylene glycol

1 | INTRODUCTION

Profitability and productivity are maintained by obtaining one calf about once a year in dairy farms. Thus, getting the cows pregnant as early as possible is critical to achieve this goal. However, decreased time of oestrus expression and poor oestrus detection seriously disrupt optimal timing of the insemination after voluntary waiting period in dairy cows (Pursley et al., 1995; Ribeiro et al., 2012). Ovsynch is the widely used fixed-time artificial insemination protocol which submit insemination of all cows without oestrus expression in dairy farms (Pursley et al., 1995) and pregnancy rates ranged from 30 to 50% (Karakaya-Bilen et al., 2019; Rabiee et al., 2005; Yilmazbas-Mecitoglu et al., 2013).

Negative energy balance (NEB) is a condition that occurs due to limited feed intake despite increased energy requirement in dairy cows and has negative effects on fertility (Manríquez et al., 2021). The limited feed intake restricts ruminal propionate for gluconeogenesis and body fat is mobilized from adipose tissue to meet this energy deficiency during the postpartum period (Locher et al., 2015; Raboisson et al., 2014). Propylene glycol (PG) is a precursor of ruminal propionate and commonly used on different days to prevent the consequences of NEB in the periparturient period (Rizos et al., 2008; Zhang et al., 2020). Drenching PG minimizes the metabolic stress with an acute effect of an increase in plasma insulin and glucose concentrations, a decrease in beta-hydroxybutyrate (BHBA) and non-esterified fatty acids (NEFA) concentrations (Butler, 2003; McArt et al., 2013; Mecitoğlu et al., 2017). Adiponectin and insulin-like growth factor-1 (IGF1) concentrations indicate the adaptive ability of cows to cope with metabolic challenges during the early postpartum period (Gobikrushanth et al., 2018; Hoedemaker et al., 2004; Krumm et al., 2017).

Several studies have been conducted on PG with different perspectives such as the usage patterns including the supplementary amount, type of compound, administration time and route and as the effects on energy metabolism, milk yield and reproductive performance in the prepartum and early postpartum periods (Lomander et al., 2012; Rizos et al., 2008). However, PG has no carry-over effect on plasma metabolites and a new metabolic adaptation period begins after ceasing PG (Bjerre-Harpøth et al., 2015). On the other hand, the effect of using an acute energy source such as PG to improve fertility during the synchronization protocol has not been investigated. In addition, it is not known how acute energy supplementation will affect fertility, especially in a period when NEB is not observed. However, based on the Lucy et al. (1992) study, drenching PG for 1 week prior to insemination may be a useful strategy to improve fertility.

Thus, the hypothesis was daily drenching PG which is administered together with the ovulation synchronization protocol, by supporting energy metabolism products that are also important for fertility, would increase the pregnancy rate. For evaluation of this hypothesis, the study was aimed to investigate the effect of daily drenching of PG during the Ovsynch synchronization protocol (days 57 ± 3 to 67 ± 3 postpartum) on BHBA, glucose, adiponectin, IGF1 concentrations, follicle size and pregnancy rate in dairy cows at first service.

2 | MATERIALS AND METHODS

2.1 | Animals, housing and management

All animal procedures were approved by the Bursa Uludag University of Animal Experiments Local Ethics Committee (Reference number: 2019/2). A total of 148 Holstein-Friesian cows (105 multiparous cows and 43 primiparous cows) were enrolled in Balıkesir, Turkey. Cows were grouped in free-stall barns and fed a total mixed ration according to their milk yield based on National Research Council recommendations (NRC, 2001). Cows were milked twice a day at 12 h interval and their access to water was ad libitum.

2.2 | Body condition score

Body condition score (BCS) was assessed using a scale (Ferguson et al., 1994) from 1 to 5, with 1 = very thin and 5 = obese at 14 days before expected calving, at the time of calving, and on the days 7 ± 3, 21 ± 3 and 42 ± 3 postpartum.

2.3 | Synchronization protocol and ultrasonographic examinations

All cows received the Ovsynch protocol on days 57 ± 3 postpartum. Gonadotropin-releasing hormone (GnRH, Gonadorelin, 50 mcg/mL, Ovarelin, Ceva, Turkey) was administered on day 0 and administration of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$, Dinoprost, 5 mg/mL, Enzaprost, Ceva, Turkey) on days 7 after the first GnRH. The second GnRH was applied 56 h later after $PGF_{2\alpha}$, and FTAI were performed 16–18 h after the second GnRH administration at postpartum days 67 ± 3.

Cows (n = 148) were randomly divided into two groups at the beginning of the Ovsynch synchronization protocol and received either PG (PG-OVS, n = 76) or water (CON-OVS, n = 72) each day of the Ovsynch protocol for first service. In PG-OVS cows received orally 300 mL of PG (Mono propylene glycol, SKC, Korea) once daily throughout the Ovsynch protocol between days 57 ± 3 and 67 ± 3 postpartum. The cows in the CON-OVS group were given the same amount of water throughout the Ovsynch protocol.

Transrectal ultrasonography of the ovaries was performed to determine follicle size and corpus luteum with a 7.5 MHz linear array probe (HASVET 838, Bursa, Turkey) at the beginning of the Ovsynch protocol and at the time of FTAI. Cows that had corpus luteum on the ovary at the beginning of the Ovsynch protocol were considered as cyclic. Follicle size larger than 10 mm on the ovary at the beginning of the Ovsynch protocol and the preovulatory follicle size at the time of FTAI were measured. The first pregnancy diagnosis was performed with transrectal ultrasonograpy on days 30 following FTAI. The second pregnancy examination was also performed to confirm pregnancy and to determine pregnancy loss on day 60 following FTAI.

2.4 | Blood sampling and analysis

Blood samples were collected from the coccygeal vein of the cows at postpartum days 7 ± 3, 21 ± 3, at the beginning of the Ovsynch protocol (days 57 ± 3) and at the time of FTAI (days 67 ± 3). The samples were centrifuged at 3000 rpm for 15 min after collection and serum samples stored at −20°C until analysis. Serum samples were used to determine adiponectin (Bovine ADP ELISA Kit, Eastbiopharm, China) and insulin-like growth factor 1 (IGF1 ELISA Kit, Eastbiopharm, China) concentrations with an specific procedure of enzyme-linked immunosorbent assay (ELISA). In both ELISA tests, samples were tested in single wells rather than duplicate wells. The serum glucose concentration was measured by the glucose oxidase method with a Fuji Dri-chem system (NX500V, Fuji Film, Tokyo). The blood BHBA concentration was also determined with a portable ketone device (β-Ketone Test Stripe, TaiDoc Technology Corp., Taiwan).

2.5 | Statistical analysis

The SPSS 23.0 software (SPSS, Chicago, USA) program was used for statistical analyses. The conformity of the data of BCS, BHBA, glucose, adiponectin, IGF1 concentrations and follicle size at different periods to normal distribution was evaluated with the Shapiro–Wilk test, and the independent sample t-test was used for comparisons between the groups (for parity [primiparous vs. multiparous] and treatment [CON-OVS vs. PG-OVS]). The chi-square test was used to compare the proportional data, and the interpretation of the results was made according to the Pearson chi-square or Fisher exact test. Statistical differences were considered statistically significant for $p < .05$ and as a tendency for $p < .10$.

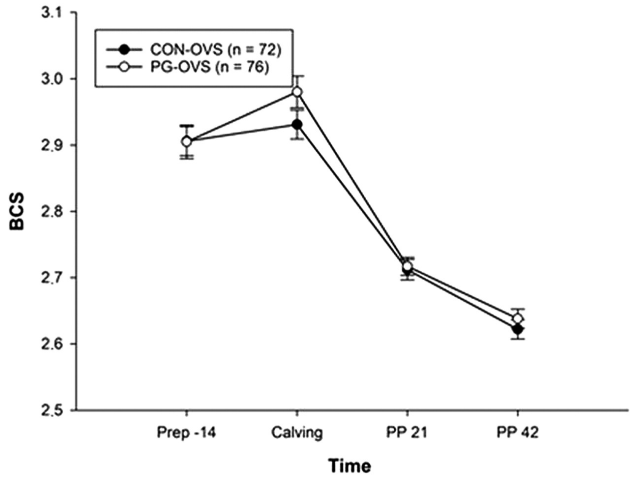

FIGURE 1 Mean body condition scores of cows of propylene glycol (PG-OVS) or water (CON-OVS) in days of periparturient period (Prep: prepartum; PP: postpartum).

3 | RESULTS

3.1 | Body condition score

BCSs were determined 14 days before expected calving, at the time of calving and on the days 21 and 42 postpartum. BCS did not differ ($p > .05$) between the treatment groups (PG-OVS and CON-OVS), as shown in Figure 1.

3.2 | Serum BHBA, glucose, adiponectin and IGF1 concentrations

The mean of BHBA concentration on day 7 postpartum was 0.68 ± 0.03 mmol/L in all cows. While BHBA concentration at the time of FTAI was lower ($p < .05$) in the PG-OVS group (0.72 mmol/L) than in the CON-OVS group (0.81 mmol/L, Figure 2), there was no difference ($p > .05$) in the mean of BHBA concentration between the groups on days 7 ± 3, 21 ± 3 postpartum and at the beginning of the Ovsynch protocol (days 57 ± 3). The distribution of individual beta-hydroxybutyric acid (BHBA) concentrations in groups are given in Figure 3.

There was no difference in serum glucose, adiponectin and IGF1 concentrations at postpartum days 21 ± 3, at the beginning of the Ovsynch protocol (days 57 ± 3) and at the time of FTAI (days 67 ± 3) between the PG-OVS and CON-OVS groups (Table 1).

3.3 | Follicle size and pregnancy rates

Cyclicity rate was similar ($p > .05$) between the groups (PG-OVS; 90.8% (69/76), CON-OVS; 90.3% (65/72)) at the beginning of Ovsynch. Follicle size (PG-OVS; 14.5 ± 0.48 mm; CON-OVS 14.3 ± 0.59 mm) was not found to be different between the groups at the beginning of the Ovsynch protocol. Preovulatory follicle size was

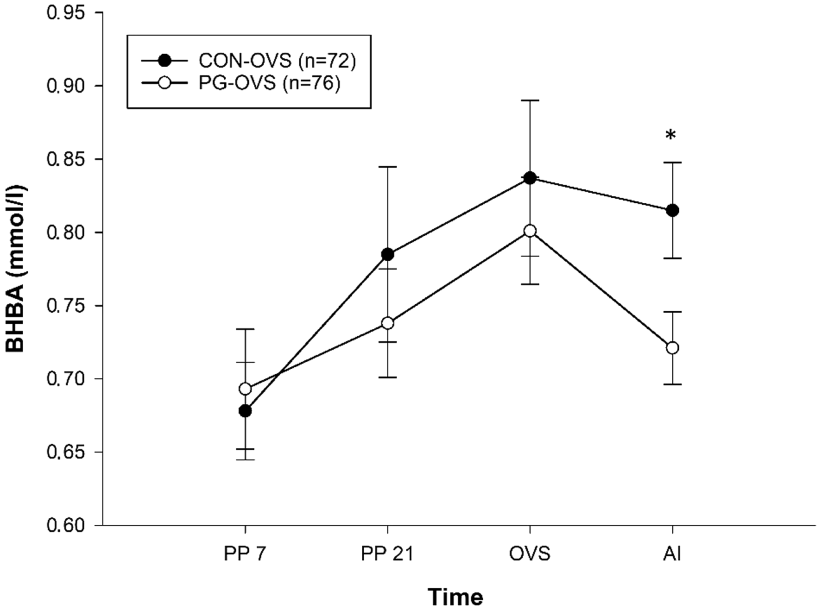

FIGURE 2 Effect of oral administration of propylene glycol (PG-OVS) or water (CON-OVS) on peripheral beta-hydroxybutyric acid (BHBA) concentrations in dairy cows during the study (PP = postpartum). *BHBA concentrations differ between the PG-OVS and CON-OVS groups ($p < .05$).

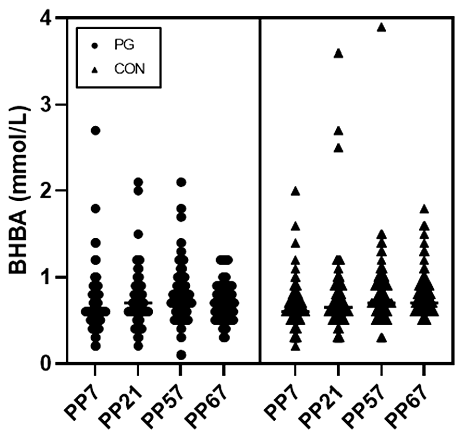

FIGURE 3 Individual peripheral beta-hydroxybutyric acid (BHBA) concentrations in the propylene glycol (PG-OVS, $n = 76$) and control (CON-OVS, $n = 72$) groups.

TABLE 1 Mean serum concentration ± SEM of adiponectin (ng/mL), IGF1 (ng/mL) and glucose (mg/dL) in cows at PG-OVS or CON-OVS groups in days of postpartum period.

	Treatments[a]		
Parameters	PG-OVS	CON-OVS	*p*
Adiponectin @21d pp	3.90 ± 0.13	4.13 ± 0.18	.32
Adiponectin @57d pp	3.70 ± 0.16	3.84 ± 0.18	.58
Adiponectin @67d pp	3.80 ± 0.16	3.77 ± 0.118	.93
IGF1 @21d pp	17.32 ± 0.77	16.73 ± 0.76	.59
IGF1 @57d pp	15.42 ± 0.70	14.84 ± 0.60	.53
IGF1 @67d pp	15.74 ± 0.71	15.77 ± 0.79	.98
Glucose @ 21d pp	68.41 ± 4.63	64.50 ± 5.47	.59
Glucose @ 57d pp	67.29 ± 3.39	72.70 ± 2.23	.18
Glucose @ 67d pp	69.11 ± 3.10	63.50 ± 3.14	.26

[a]Oral daily administration of propylene glycol (PG-OVS) or water (CON-OVS) during the Ovsynch protocol in lactating dairy cows.

also similar at the time of FTAI with 17.8 ± 0.52 mm in the PG-OVS group and 17.7 ± 0.42 mm in the CON-OVS group ($p > .05$).

Pregnancy rate was higher ($p = .05$) in the PG-OVS group than in the CON-OVS group on day 30 following FTAI (Table 2). Pregnancy rate in the PG-OVS group tended to be higher ($p = .07$) than in the CON-OVS group on day 60 after FTAI and pregnancy loss were found similar between the groups (Table 2). Pregnancy rate was affected by parity on day 30 after FTAI. Pregnancy rate in the PG-OVS group (49.1%, 27/55) were higher ($p < .05$) in multiparous cows than in the CON-OVS group (30.0%, 15/50), a similar effect was not observed in primiparous cows.

4 | DISCUSSION

The daily drenching of oral PG during the Ovsynch protocol did not change the glucose, IGF1 and adiponectin concentrations in our study. However, it was determined that drenching of PG throughout the Ovsynch protocol decreased the BHBA concentration in the PG-OVS group at the time of FTAI compared to that in CON-OVS. Likewise, Butler (2003) reported lower systemic concentrations of both NEFA and BHBA in lactating dairy cows that drenched with PG. Consistent with the findings of the previous study, it was found that drenching of oral PG throughout the Ovsynch protocol was effective to reduce serum BHBA concentrations at the time of FTAI, which had a positive effect on pregnancy rate in this study. It is important to note that the relationship between BHBA concentrations and fertility is complex and may vary depending on the individual cow and the specific circumstances. It has been demonstrated that higher BHBA concentration impaired hypothalamic pituitary–ovarian axis, granulosa cell development, follicular steroidogenesis in granulosa cells and oocyte maturation (Butler, 2003;

TABLE 2 Effect of daily administration of propylene glycol during the Ovsynch protocol on pregnancy rate in the lactating dairy cows.

	Treatments[a]		
	PG-OVS	CON-OVS	*p*
Pregnant, % (*n*/*n*)			
Days 30	46.1 (35/76)	30.6 (22/72)	.05
Days 60	42.1 (32/76)	27.8 (20/72)	.07
Pregnancy loss, % (*n*/*n*)	3.9 (3/76)	2.8 (2/72)	.69

[a]Oral daily administration of propylene glycol (PG-OVS) or water (CON-OVS) during the Ovsynch protocol in lactating dairy cows.

Gong et al., 2022; Vanholder et al., 2015). On the other hand, a decreased BHBA concentration can have a positive effect on steroid mechanisms, increasing the quality of oocytes and the ability of embryo development in dairy cows (Ospina et al., 2010; Pishvaei et al., 2021). Therefore, when BHBA concentrations decrease, it can improve the function of follicular steroidogenesis and promote normal reproductive processes in dairy cows. Pishvaei et al. (2021) reported that in their study based on the Ovsynch protocol, a higher pregnancy rate was indicated in the cows with a lower BHBA μmol/L concentration (380 ± 0.15 compared to 540 ± 0.26) during the early postpartum day. The study also showed that cows with lower BHBA concentrations had higher oestrous symptoms at insemination because of higher oestradiol and lower progesterone concentrations (Pishvaei et al., 2021). In our study, since a timed AI synchronization protocol was used, neither oestrous symptoms nor oestradiol concentration were observed, but it is thought that the significant increase in pregnancy rate in the PG-OVS group might be related to the positive effect on steroid mechanisms. On the other hand, improving energy metabolism may contribute to hypothalamic responsiveness to circulating oestradiol, resulting in increased GnRH pulse frequency (Butler, 2003). It is unknown whether the decreasing BHBA concentration is directly responsible for the support of the hypothalamic responsive and concomitant improvement in LH pulse, but this is another possibility. Walsh et al. (2007) reported that for every 100 μM increase in BHBA concentration in the postpartum weeks 1 and 2, there was a 2% and 3% decrease pregnancy rate after the first AI in cows, respectively. Similar to our results, pregnancy rate was 13% higher in cows that had lower (<1.0 mM) BHBA concentration within 70 days after the end of the voluntary waiting period compared to cows that had higher (≥1.0 mM) BHBA concentration (Ospina et al., 2010). Also, other studies have not been able to fully elucidate the exact mechanism of fertility increase with low BHBA concentrations, similarly, this study showed that administration of PG during the Ovsynch protocol decreased BHBA concentrations and resulted in higher P/AI in the PG-OVS group.

In our study, while BHBA concentrations were significantly lower in the PG-OVS group compared to the CON-OVS group, there was no significant difference in blood glucose concentrations between the groups. This may be related to the time of blood collection. Blood samples were collected approximately 4–6 h after the morning feeding in order to increase the possibility of detecting possible elevated BHBA concentrations (Panousis et al., 2017) and PG was applied as a drench after blood collection. The fact that the half-life and elimination rate of blood glucose is approximately four times higher than that of BHBA, can be explained as the reason why glucose concentrations were similar between the groups, while the difference between BHBA concentrations was determined at the time of sampling (Abuelo et al., 2016).

Insulin and IGF1 play a role in the proliferation and steroidogenesis of granulosa cells by increasing the effects of pituitary gonadotropins on ovarian steroidogenic cells (Miyoshi et al., 2001). Similarly, the concentrations of IGF1 in serum and follicular fluid is positively related to energy balance and positively affect follicular development process in cows (Hoedemaker et al., 2004). Lucy et al. (1992) showed that a short-term change in energy balance affects the growth of the preovulatory follicle. Also, Miyoshi et al. (2001) reported that drenching of 500 mL PG between postpartum days 7 and 42 could be a useful alternative strategy on fertility by increasing the preovulatory follicle size and pregnancy rate in the first insemination. However, in the present study, there were no differences neither in IGF1 concentration nor on preovulatory follicle size.

Adiponectin is an adipocyte-derived hormone that regulates energy metabolism by stimulating fatty acid oxidation (Lord et al., 2005; Mecitoğlu et al., 2016). Measurement of adiponectin in blood can provide information about the adaptive ability of cows to cope with metabolic challenges. It was reported that reduction in adiponection concentration by an average of 21% from prepartum to early postpartum period was partly associated with NEB. Increment of adiponection concentration occurs independent of loss of adiposity after postpartum period (Krumm et al., 2017). Adiponectin concentration did not differ between the groups and on different postpartum days in our study.

Several modifications including hormonal applications have been conducted to increase fertility in the Ovsynch protocol (Keskin et al., 2010; Yilmazbas-Mecitoglu et al., 2013). However, the effect of administration of an acute energy-providing preparation such as PG during the synchronization protocol on fertility has not been evaluated. The most important finding of this study was significant decrease in blood BHBA concentration at the time of FTAI in the Ovsynch protocol had a positive effect on fertility.

5 | CONCLUSION

Drenching of daily oral PG during the Ovsynch protocol did not change serum glucose, IGF1, adiponectin concentrations. However, supplementation of PG to the Ovsynch protocol decreased serum BHBA concentration at the time of FTAI and increased the pregnancy rate in dairy cows. Comprehensive studies are needed to reveal the acute effect mechanism of PG administration on pregnancy rate in the synchronization protocol.

AUTHOR CONTRIBUTIONS

Rabia Cakircali handled conceptualization, methodology, investigation and writing original draft. Ebru Karakaya-Bilen, Baris Guner and Cihan Tolga Ortac handled interpretation, writing original draft, review and editing of the manuscript. Abdulkadir Keskin and Zafer Mecitoglu handled interpretation, review and editing of the manuscript. Abdulkadir Orman statistically analysed the data. Ahmet Gümen handled conceptualization, methodology, writing, review and editing of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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