Endocrine Changes Associated with Ovarian Activity in Montbéliarde Cows Raised Under Semi-arid Condition in Eastern of Algeria

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Abstract: The objective of the current study was to investigate changing profile of reproduction hormone (Progesterone and Estradiol) BCS and parity in relation to the resumption of ovarian cyclicity postpartum in cows of European origin under semi-arid conditions of Algeria. To measure plasma progesterone (P4) and estradiol 17-β (E2), blood samples were collected from 21 Montbéliarde dairy cows at 30, 40 and 50 d postpartum (dPP) respectively. Body condition score (BCS) was taken before and after calving. Cows were grouped based on progesterone concentration (resumption of ovarian cyclicity, ≥ 1 ng/mL) at 30, 40, and 50 dPP into a non ovarian activity (NOA) group (n = 6) and ovarian activity (OA) group (n = 15). P4 concentration was higher in the OA group than in the NOA group and statistically differed at 50 dPP (p = 0.04). E2 was higher in the OA group than a NOA group at d 30 (p = 0, 01) and d 40 (p = 0, 03). Despite superiority of BCS peri and postpartum but no significant differences were detected between the two groups. There was no significant difference between parity and ovarian resumption groups. Two groups were formed based on differences in onset of postpartum resumption of ovarian activity. OA (n =15/21 or 71.42 %) showed first ovulation between 30 d and 50 d after parturition. NOA (n = 6/21 or 28.57 %) manifested a first ovulation > 50 d in milk.

Keywords: Cow, Montbéliarde, progesterone, BCS, parity, luteal activity, postpartum, Algeria.

INRODUCTION

Livestock policies requested to reduce reliance on milk import and its by-products.

Algeria imports annually thousands of pregnant dairy heifers mainly from Europe especially Montbéliarde breed. Many reproductive problems and namely extended calving to conception interval reduce herds' profit. Profitability of dairy cattle herds is tightly linked with reproduction. The ideal goal to be achieved being one calf per cow per year [1]. Postpartum ovarian activity need to rebound within a calving conception interval compatible with herd profitability and within an optimal biological period. Progesterone measurements have been used in cattle reproduction for more than 30 years for diagnosis of pregnancy, monitoring of ovarian activity postpartum, assessing the reproductive status of the cow [2]. The effect of BCS during the pre- and postpartum periods, on reproductive parameters was recorded. Kafi and Mersaei [3] showed that loss of BCS ≥ 0.75 points during 49 days after calving increased the risk of delayed first ovulation. In addition, previous research indicated that ovulatory cows had a higher BCS than anovulatory cows [4]. However, other studies found no difference in BCS between the different groups of ovarian cyclicity during pre- and postpartum periods [5].For the parity variable, bibliographic research reported controversial results. Several researchers observed that some authors report the relationship between parity and days from calving to first ovulation was significant among primi-, bi- and multiparous dairy cows under similar good body nutritional conditions during postpartum period [6].Therefore, other studies reported no difference in mean age among different ovarian resumption groups [7].

Few studies documented the postpartum cyclicity in Montbéliarde cow compared with other breed (Holstein, Limosin, Friesian…). More, the use of hormones in postpartum reproductive management of dairy cows is still not well practiced in Algeria. For these reasons, the aim of the present study was to determine the relationship of E2, BCS peri and postpartum and parity and subsequent ovarian activity performance of Montbéliarde cows in Algeria.

MATERIALS AND METHODS
Animals, Housing, Diets and Management

The experimental was conducted from October 2017 to February 2018 on Montbéliarde dairy cows in a pilot farm specialized in livestock service (COOPSSEL) in Setif (latitude 36°11'N and longitude 5°24'E, 1100 m above sea level). 21 Montbéliarde dairy cows were used in this study divide in two groups, 9 at second and 15 at third parity, in good body condition at dry period (3.52 ± 0.09). The cows selected for the study were maintained under loose housing system in clean and hygienic paddock. All the animals were fed a total mixed ration ad libitum consisting of grass and concentrate twice a day. *Ad libitum* fresh drinking water was available throughout the day postpartum. Cows were diagnosed with one or more pathological event, including dystocia, retained placenta, mastitis, calving difficulty, uterine infection and general reproductive problems were excluded from the study. Nutritional status can be appreciated by BCS which is an accepted non-invasive, subjective, quick, and inexpensive method to estimate the degree of fatness. All BCS measurements were done by a trained single operator for a given herd utilizing both visual and tactile technique. Animals were classified as described by [8].

Study Design

The cows were divided into two groups according to the return of luteal activity during the postpartum period based on the progesterone concentration (resumption of luteal activity, ≥ 1 ng/mL [9-11] at 30, 40, and 50 dPP. Cows were grouped in to groups. Cows with resumption ovarian activity (OA) and cows without resumption ovarian activity (NOA). The group OA had plasma progesterone concentration ≥ 1ng/mL at each sample time points, whereas the group NOA had progesterone concentration < 1 ng/mL from the sample time points. Uterine involution was complete at 30 dPP for all cows.

Blood Sampling Protocol

Blood samples were collected by coccygeal venipuncture (peripheral blood) at 30, 40 and 50 dPP. Needles used were 18Gx1$^{1/2}$ (1.2x38mm; AGANI^{TM} Needle) to minimize stress during sample collection. Four milliliters of blood were collected into lithium-heparin tubes. Samples were kept inside the isothermal box (4°C) until transported to the laboratory where they were analyzed. All procedures were approved under convention of Ferhat Abbas University Setif-1 with the farm (COOPSSEL).

Reproductive hormones Assay
Progesterone assay

Plasma progesterone concentrations were determined by Chimiluminescent Immuno Assay used competitive immunoassay with analyte liberation using a commercial kit (Progesterone ROCHE Diagnostic) and the automate analyser COBAS E-System (e-411). According to ROCHE Diagnostic (2016), 30 µL of the sample is incubated with a biotinylated, monoclonal progesterone-specific antibody, ruthenylated progesterone derivative, and danazol (to release progesterone). The antibody binding sites are occupied by either progesterone or the ruthenylated derivative, with the proportion of each depending on the concentration of progesterone in the sample. The sensitivity of the test, defined as the least detectable concentration of progesterone (2 S.D. away from the zero of dose response), was 0.095 nmol/L (0.030 ng/mL). The recovery rate of the assay ranged from (0.030 - 60.0 ng/mL). A standard curve was developed with concentrations of progesterone and the intra-and inter assay coefficients of variation were 3.7 and 5.5% respectively.

Estradiol assay

According to ROCHE Diagnostic (2016), 25 µL of the sample is incubated with two Estradiol (E2)-specific biotinylated antibodies and mesterolone that liberates protein-bound E2. Chemiluminescence is then induced by applying a voltage and measured with a photomultiplier. The signal yield is roughly reversely proportional to the total E2 concentration in the sample. Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode. Measuring range was 5-3000 pg/mL and the intermediate precision was COBAS E-System (e-411) analyzer: 2.5-11.9% (25.4-2,932 pmol/L). The sensitivity of the test was 18, 4 - 15781 pmol/L (5, 0 - 4300 pg/mL).

STATISTICS ANALYSES AND DATA MANAGEMENT

The data were analyzed using SPSS program (Statistical data software Package for Social Scientists analysis, version 21). The changes of mean plasma of E2 and P4, the incidence of peri-and postpartum BCS, cow parity (second and third)was compared between the groups using One way ANOVA. Tukey's test was used to detect statistical differences between the mean values of BCS and hormonal in different categories of cows based on postpartum P4 profile. The post hoc LSD test was used to evaluate the statistical significance of differences between group means. Pearson's coefficient was used to analyze a possible correlation between the different variables and ovarian

activity resumption at specific time points post calving. The data are presented as the mean ± standard deviation. For all tests, statistical significance was accepted at $p < 0.05$.

RESULTS AND DISCUSSION
Hormone Data

The difference in the mean plasma concentrations of P4 between the two groups was significant at 50 dPP ($p = 0.04$). The profile of P4 in OA showed a decrease among sampling times (30, 40 dPP) and an increase between 40 and 50 dPP. NOA showed an increase plasma level of P4 between 30 and 40 dPP then a decrease between 40 and 50 dPP (Table 1 and Fig1). Day of sampling had no significant effect on plasmaP4 concentrations in OA and NOA ($p = 0.93$; $p = 0.91$ respectively). The plasma concentrations of E2 in ovulatory cows were higher than those in anovulatory cows among sampling times. However, there were no significant differences between groups at 50 dPP. The profile of plasma estradiol levels was similar for both OA and NOA groups. It is characterized by a decrease at 30 and 40 dPP then an increase between 40 and 50 dPP (Table 1 and Fig1). E2 concentrations were not differed across the sample times in OA ($p = 0.80$) and in NOA ($p = 0.56$).

Table-1: Postpartum Plasma Hormonal (mean ± SE) in the OA and NOA Cows.

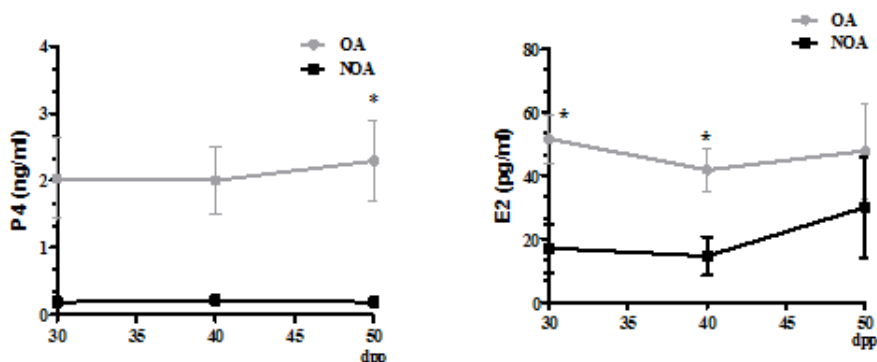| dPP	Groups	P4 (ng/ml)	P-Value / LSD	E2(pg/ml)	P-Value/ LSD
30	OA	2.03 ± 0.6	0.1 / 2.68	51.61 ± 7.80	0.01 / 33.42
	NOA	0.19 ± 0.05		17.07 ± 7.62	
40	OA	2.00 ± 0.5	0.05 / 2.18	41.75 ± 7.02	0.03 / 29.68
	NOA	0.22 ± 0.06		14.56 ± 6.08	
50	OA	2.29 ± 0.60	0.04 / 2.45	47.84 ± 15.02	0.5 / 65.01
	NOA	0.19 ± 0.06		29.96 ± 15.89	

Fig-1: Circulating concentrations of plasma P4 and E2in OA and NOA cows at 30, 40 and 50 dPP

It is well known that reproductive performance of dairy cows is influenced by the ability to resume estrous cycles early postpartum [12]. The present study examined the relationship among postpartum various hormonal (P4 and E2), dynamique change of BCS peri and around calving and parity at a specific time postpartum in Montbeliard dairy cows raised under semi-arid climate that may be indicative for reestablishment of ovarian activity. What would be the hormonal, BCS profile in relation with luteal activity early postpartum? Our results illustrated in table 1 and figure 1shown that higher plasma E2 concentrations as measured in peripheral plasma was unregistered in OA group compared with those of the NOA cows which are well confirmed the previous studies of [13-15]. However, there were no significant differences at 50 postpartum because an increase of preovulatory E2 concentrations. In our work, we can justify a low levels of peripheral estradiol in NOA cows may be indicate that development of dominant follicle occurred independently from steroidogenic activity during the early postpartum period; and the failure ovulation in NOA was associated with low levels of E2. This finding may be explained by an insufficient ability of the granulosa cells to secret E2 [15] or in other hand, an important factor of metabolic hormonal such as IGF-I, GH and insulin that are not measured in this study may be important to follicular development and maturation in postpartum, ovulation and granulosa cells steroidogenesis [13,14] or due in part to lower LH pulsatility and lower intrafollicular concentration of androstenedione and estradiol [15]. In the present study, plasma concentration of P4 in cow with OA was higher and statistically significant than NOA during 50 dPP. However, Kafi *et al.* [9] reported that the concentration of P4 in cows with prolonged luteal phase (PLP) were higher than in normal luteal phase, indicating that the occurrence of PLP in the clinically healthy dairy cows was not related to uterine infection. Cheong *et al*. [15] found that prepartum circulating plasma P4 concentrations declined over time (day from calving) with no difference between groups. In addition, P4

concentration in the follicular fluid was not significantly between groups.

Relationships among ovarian activity and BCS peri- and postpartum

No difference in BCS was detected between groups at specific time peri- and postpartum. The BCS tended to be higher in the OA group compared with NOA. At 3 week after calving (3.61 ± 0.11 *vs* 3.29 ± 0.11 respectively; p = 0.10), at 30 dPP (3.16 ± 0.13 *vs* 2.83 ± 0.19 respectively; p = 0.17), at40 (3.12 ± 0.12 *vs* 2.71 ± 0.15 respectively; p = 0.07) and at 50day before calving (3.26 ± 0.10 *vs* 2.87 ± 0.14 respectively; p = 0.05) (Fig-2). Although BCS change was differed across the sample times in OA (p = 0.01), but not in NOA (p = 0.06).
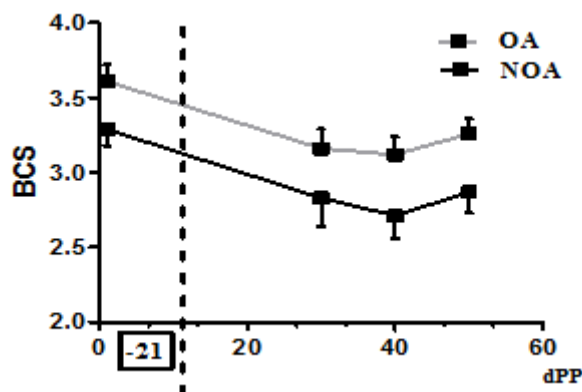

Fig- 2: BCS in NOA and OA cows at 3weeks prepartum, and at 30, 40 and 50 dPP.

We evaluated relationships between postpartum ovarian activity and energy balance using a BCS measurement. The present study could not examine the exact nutriment intake and nutritional status, because all cows were provided with feed and water available *ad libitum*. Our data showed the dynamique change of BCS peri and postpartum in both groups and found that OA and NOA cows have no significatif difference despite superiority of BCS in the cyclical group in peri and postpartum(Fig 2).The same result was reported by Kalem *et al*. [11] who found no relationship between BCS at 0, 15, 30, 41and 52 dPP in early responders and late responders of Montbeliard cows in Algeria suggesting in pre and post calving stage, the similarity of food systems explains the low variability of BCS and the similar diet convenient in Algeria. In addition, body condition at the time of calving did not affect cyclicity [16]. Other studies show no association between BCS during pre- and postpartum periods and resumption of postpartum cyclicity [17]. Conversely, Jeong *et al*. [10] reported that the BCS tended to be lower in the non-cycling group at 4 weeks postpartum (P < 0.1) and was significantly lower at 6 weeks postpartum (P < 0.001).

Our data found effect of time in BCS change in cycling group (p = 0.01) and no effect in NOA (p = 0.06). However; OA group mobilize little fat stores than NOA (BCST-BCS 30, 0.45 *vs* 0.46 respectively); (BCST-BCS 40, 0.49 *vs* 0.58 respectively); (BCST-BCS 50, 0.35 *vs* 0.42 respectively) suggesting that OA is able to handle better the negative energy balance (NEB) after parturition when compared with anovulatory cows. In this direction cows with greater NEB have lower peripheral concentrations of IGF-I and LH, which act synergistically to promote ovarian follicular development, also lower plasma progesterone concentration and no-functional corpora lutea in the postpartum cows [7].Whereas, Jeong *et al*. [10] found an effect of sampling time in BCS (p < 0.0001) in cycling and non- cycling group, so Kalem *et al*. [11] Reported no effect of sampling period in BCS change in both group (p = 0.13).

Relationships among ovarian activity and parity

The interaction between cow parity and sampling time on plasma hormonal and BCS was not significantly different (Table 2).

Table-2: Interaction among ovarian activity and parity and different variables

dPP	Variables	F	P
30	P 4	0.17	0.68
	E2	2.11	0.16
	BCS	1.13	0.30
40	P 4	0.30	0.53
	E 2	0.07	0.78
	BCS	0.46	0.50
50	P 4	2.20	0.15
	E 2	0.60	0.44
	BCS	1.18	0.29

Data between parity according to ovarian activity category was summarized in fig3**.** BCS peripartum was not differed in both group (3.87 ± 0.15; 3.41 ± 0.22; $p = 0.13$). BCS in cows with OA were more than those in cows with NAO at postpartum period; but no differences were detected. Profile of P4 had shown no significant difference. The mean E2 in cows with NOA was lower than that in cows with OA at day postpartum; however, difference was observed between groups at 40 day after calving ($p = 0.009$).
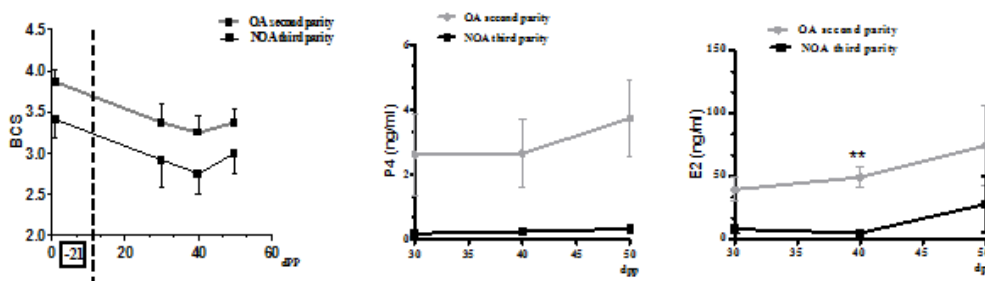

Fig- 3: BCS and circulating concentrations of plasma P4 and E2in OA and NOA for each time postpartum and for each parity group

Data were analyzed by dividing into two groups: OA third parity ($n = 9$) and NOA second parity ($n = 3$). There were no differences in prepartum BCS between nonovulatory second parity and ovulatory third parity cows (3.16 ± 0.08 *vs* 3.44 ± 0.13 respectively; $p = 0.26$).BCS postpartum did not differ significantly among groups. There was no difference among ovarian resumption groups for P4 and E2 levels until period study (Fig-4).
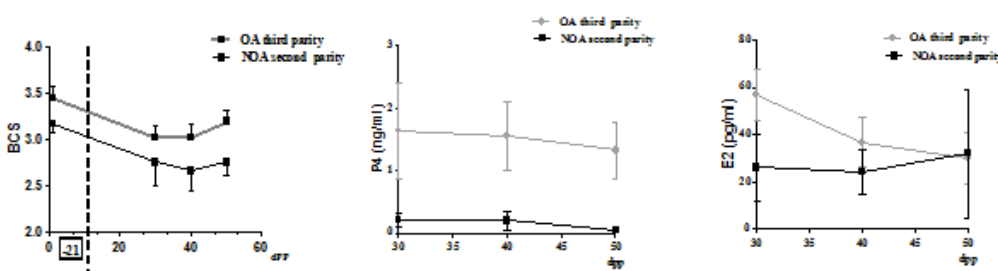

Fig-4: BCS and circulating concentrations of plasma P4 and E2in OA and NOA for each time postpartum and for each parity group

The parity of the cow has not always been considered, which may have masked some potentially significant relationships. It is possible that the influence of parity on the resumption of ovarian cycle is modulated by the factors different from the nutrition-related changes during the postpartum period in dairy cows. To provide credible estimates of parity, the data collected showed no significant effects of interaction between parity and luteal activity for all cows which it was similar to previous reports [18]. In addition, there was no difference in mean age among different ovarian resumption groups [7]. Our finding are not compatible with study of Tanaka *et al*. [6] who demonstrated that the influence of parity on days from calving to first ovulation was significant among primi-, bi- and multiparous cows under similar good body nutritional conditions.

Resumption of ovarian activity

21 cows which were used in the present study, 71.42 % (15/21) had resumption of postpartum ovarian cyclicity and showed first ovulation within 50 dPP. NOA (n = 6/21 or 28.57%) were cows with a first ovulation > 50 day after calving. In second parity (n =

9), of the 21 cows, 3/21 (14.28 %) was resumed ovarian cyclicity within 30 days after calving, and 3/21(14.28 %) until 40 and 0/9 until50 days after calving. Third parity (n = 12), only 3/21(14.28%) of the cows resumed ovarian cyclicity at 30 and 40 dPP (14.28%) and at 50 dPP 3/21 (14.28%) (Fig- 4).
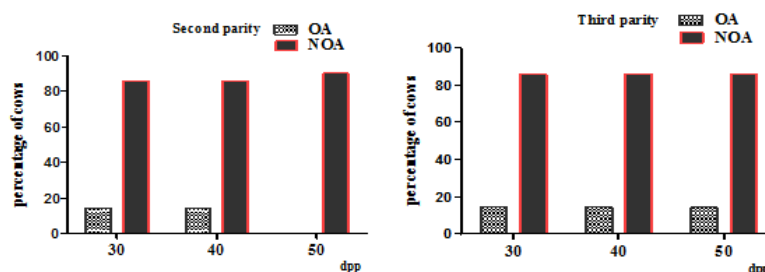

Fig-4: Percentage of cows that resumed ovarian cyclicity postpartum at different postpartum period and parity

The resumption of ovarian activity seemed relatively higher in the present study (71.42 %) than some earlier report (32.6%) from the same breed in France [20] and 56% from the same breed in Algerian [11]. This percentage of return on ovarian activity from Montbeliard breed recorded in the present study can be explain by a better reproductive performance express functional adaptation for the new environment of Algerian. The incidence of NOA obtained in our data was (28.57 %), however, in previous studies was 28.8% [9], 33.3% [21] and 44% [11]. Previous studies based on milk progesterone profiles reported that second parity and third parity had 61.5 % and 32.2 % resumption of ovarian activity postpartum [18]. In the current study, it was 28.56 % and 42.84 % respectively. These above differences in percentage of cows that resumed or not ovarian activity in postpartum period may be suggesting by possible reasons contributing to discrepancies among the studies include breed difference, the sample population analyzed (number of animals and variation in the parameters within the sample population), production level, nutritional status and management including the frequency of BCS measurement, the model of analysis, reproductive parameters investigated, parity, calving season, production systems (extensive or semi-extensive), breeding conditions and environmental factors.

Correlation among hormones, BCS and parity and luteal activity

Pearson correlations coefficients were used to postulate relationships between luteal activity and variables that describe plasma concentrations of P4 and E2 and other variables (BCS and parity). A strong positive correlation was found (P = 0.001) between P4 at 50 dPP and E2 at 50 dPP. BCS peripartum was positively correlated with BCS post calving. The P4 levels at 50 dPP were correlated with parity (r = -0.51, p = 0.04) (Table 3). Our data confirmed the previous studies that correlation between BCS and parity was small but negative [19].

Table-3:Associations (correlation coefficients) among measures of luteal activity plasma hormones, BCS and parity in postpartum Montbeliard dairy cows at d 30, d 40 and d 50 postpartum

	P 4 30	P 4 40	P 4 50	E2 30	E2 40	E2 50	BCST	BCS 30	BCS 40	BCS 50	parity
P 4 30		-0.01	-0.18	0.37	0.20	0.10	0.39	0.22	0.24	0.38	-0.18
P 4 40			-0.07	-0.26	-0.28	-0.13	-0.34	-0.32	-0.28	-0.06	-0.26
P 4 50				-0.12	0.17	**0.76*****	0.42	0.46	0.05	0.40	**-0.51***
E2 30					0.38	-0.11	0.30	0.28	0.008	**0.58***	0.33
E2 40						-0.02	0.34	0.19	-0.08	0.38	-0.23
E2 50							0.24	0.36	0.16	0.44	-0.38
BCST								**0.78*****	**0.58***	**0.55***	-0.50
BCS 30									**0.62***	**0.70****	-0.34
BCS 40										0.39	-0.23
BCS 50											-0.21
Parity											

CONCLUSION

The present study demonstrated that hence early postpartum P4 rise and circulating estradiol concentrations, superiority of BCS peri and postpartum were associated with increased resumption of ovarian activity. No effect of parity or change of BCS was related to the anovulatory conditions. It is recommended a routine monitoring BCS of cows before and after calving would help identify cows with poor nutritional status and improve nutritional management; should increasing the proportion of cows that resume normal ovarian cycle and increase reproductive efficiency. More, associated P4 and E2 measurements with other plasma components (hormones and metabolic profiles), may help to provide appropriate management to promising strategy for improving fertility after parturition and emphasized to ameliorate reproductive performance of Montbéliarde dairy cow in semi-arid conditions of Algeria.

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