Revised

Melatonin concentrations in the two jugular veins, and relationship with the seasonal reproductive activity in goats

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Abstract

We investigated whether melatonin concentrations vary between the two jugular veins and whether absolute (nocturnal) or relative (nocturnal/diurnal ratio) plasma melatonin concentrations are associated with seasonal reproductive activity measured by oestrous or ovulatory activity in Payoya goats. Thirty-two adult Payoya goats were penned under natural photoperiod. Oestrous activity was tested daily using aproned males — twice a week plasma was sampled for progesterone. Melatonin plasma concentrations were studied at each equinox and solstice of the year in jugular samples taken simultaneously by venipuncture. Nocturnal and diurnal plasma melatonin concentrations from each jugular vein were assessed in 3 and 2 plasma samples per goat, respectively, taken at hourly intervals in each period. No differences in melatonin concentrations between the two veins were observed, but there was a significant interaction ($P<0.001$) between jugular vein and animal in nocturnal melatonin concentrations. There was no effect of sampling period on melatonin concentrations and the coefficient of correlation between sampling periods was very high. The analyses performed indicated that neither absolute nor relative melatonin concentrations were related with the dates of onset or end of ovulatory/oestrous activity. Therefore, we concluded that in goats (1) melatonin concentrations are highly variable between jugular veins in the same individual but not in the general population, (2) melatonin concentrations are highly repeatable for each individual, and (3) absolute and relative amplitudes of melatonin concentrations are not linked to the seasonal breeding activity in Mediterranean goats.

Key words: Goat, melatonin, jugular, seasonality, reproduction.

1. Introduction

Reproductive activity in goats shows a clear annual pattern, with a period of breeding activity that begins in the late summer or autumn, when daylight is decreasing, and the onset of seasonal anoestrus in late winter or at the start of spring, with increasing daylight. This seasonal breeding cycle in goats is not directly driven by the photoperiod, rather it appears to be a reflection of the expression of a self-sustained endogenous rhythm that is synchronised or entrained by the photoperiod [1]. The reproductive response to photoperiod is mediated by the pineal gland via changes in the daily secretion of its main secretory product, melatonin [2] that is read by a large variety of tissues in the whole organism [3].

After its synthesis, melatonin is secreted via the vasculature of the pineal that flows into the vein of Galen or vena cerebri magna. This cerebral vein drains into the sinus rectus, and venous blood is delivered to the internal jugular veins via the lateral sinus [4]. An intriguing feature of melatonin concentrations in the blood is the existence of a high variability between individuals, as has been reported in sheep. A major component of this inter-individual variability is the size of the pineal gland [5]. Other components could originate from within-individual differences in melatonin concentrations between the two jugular veins, and/or random sampling one of the two jugular veins, as has been demonstrated in sheep [6].

Two main alternative hypotheses have been proposed to explain how nycthemeral melatonin secretion controls reproductive activity: the duration of the melatonin secretion, and the temporal phase of this rise relative to the light-dark cycle have both been considered [7-10]. For each hypothesis, the amplitude of melatonin secretion may play a specific role to modulate the response; it has been suggested that the ratio nocturnal/diurnal melatonin after implant insertion [11] or under natural conditions [12] could be related to the speed of resumption of ovulatory activity. A significant relationship between the mean nocturnal melatonin concentration and the intensity of seasonal breeding activity has also been reported in the female Italian buffalo [13]. Those authors observed that animals showing less tendency to seasonality presented lower levels of melatonin concentration during the night. However, Zarazaga et al. [14] have shown that neither absolute nor relative amplitude of melatonin concentrations is linked to the seasonal breeding activity in ewes. The literature contains very few results on the existence of a relationship between absolute or relative melatonin concentrations and seasonality in goats.

Thus, in the present study, we used goats to determine (1) whether there is a difference in melatonin concentrations between jugular veins sampled at different moments of the year and (2) whether the dates of onset and end of the annual breeding season are linked to the melatonin concentrations.

2. Material and methods

2.1. Animals and management

This experiment was performed in accordance with the Spanish Animal Protection Policy RD1201/2005, which conforms to the European Union Directive 86/609 regarding the protection of animals used in scientific experiments.

The study was conducted at the experimental farm of the University of Huelva (latitude 37º 15’), which meets the requirements of the European Community

Commission for Scientific Procedure Establishments (1986). Thirty-two adult and non-pregnant Payoya goats, which had kidded at least 5 months previously, were used.

Throughout the experimental period, the goats were kept permanently in communal yards with an uncovered area, and without any supplementary light. The animals were maintained under intensive management and were fed daily with lucerne hay, barley straw *ad libitum*, and commercial concentrate, according to INRA standards [15], to maintain adult weight. All animals had free access to water and mineral blocks containing trace elements and vitamins.

Oestrous activity was tested daily using entire aproned males. Females standing at mounting by the male were considered in oestrus. In order to determine the onset of ovarian activity, blood samples were collected twice a week from each animal by jugular venipuncture and assayed for progesterone. Immediately after collection, samples were centrifuged and the plasma was stored at –20 ºC until assay.

2.2. Sampling for melatonin determination.

Nocturnal and diurnal melatonin plasma concentrations of the goats were assessed at each equinox and solstice of the year (“sampling periods”) (spring equinox: 20th March, summer solstice: 21st June, autumn equinox: 23rd September, and winter solstice: 21st December). Nocturnal melatonin plasma concentrations of goats were assessed in three plasma samples of each jugular vein, taken at hourly intervals during the night, starting 3 hours after sunset (“time of collection”). Blood samples from the two jugular veins were obtained by venipuncture in evacuated tubes with heparin by two operators to ensure the simultaneity of sampling, and special care was taken to puncture precisely at the same level of the neck on the two sides. All goats were always sampled within 15-20 min, and the same order was maintained at each sampling time. Blood samples were collected under dim red light (less than 1 lux at 20 cm), avoiding any direct illumination of the eyes. Diurnal melatonin plasma concentrations were assessed in two plasma samples of each jugular vein, taken at hourly intervals, starting 4 hours after sunrise. Plasma was immediately separated by centrifugation and stored at -20 ºC until assay.

2.3. Definitions of reproductive activity.

Ovulatory activity was confirmed when four consecutive plasma samples had progesterone concentrations above baseline (1 ng/mL) with subsequent cyclicity. For each goat, the date of the last plasma progesterone value below baseline that was continued by the first extended cyclic pattern was taken as the onset of ovulatory activity. Ovulatory activity was considered to have ceased when five or more consecutive plasma samples had concentrations below baseline. The date of the last plasma progesterone value below baseline at the completion of the last extended cyclic progesterone pattern was taken as the end of ovulatory activity.

The mean duration of oestrous cyclicity and the ovulatory season were defined as the number of days between the first and the last detected oestrus or the first and the last ovulation, respectively, in the same breeding season [16].

2.4. Hormone assays.

Plasma progesterone concentrations were assayed by radioimmunoassay using the technique described by Terqui and Thimonier [17]. The sensitivity of the assay was

0.125 ng/mL. The intra- and interassay coefficients of variation were 7.6% and 8.7%, respectively.

Plasma melatonin concentrations were measured in duplicate aliquots of 100 microlitres of blood plasma by radioimmunoassay, using the technique described by Fraser et al. [18], with antibody first raised by Tillet et al. [19]. The sensitivity of the assay was 4 pg/mL. The intra- and interassay coefficients of variation were 18.8% and 14.2%, respectively.

Hormone assays were carried out at the Assay Laboratory of the Station de Physiologie de la Reproduction et des Comportements (INRA, Nouzilly, France).

2.4. Statistical analysis.

Absolute and relative (night/day ratio) plasma melatonin concentrations per jugular and season were calculated for each goat. Night or day plasma melatonin concentrations were analysed first by a repeated-measure ANOVA to assess a possible interaction between “jugular side” and “time of collection” for each “sampling period”. The objective of this analysis was to assess a possible interaction between “jugular side” and “time of collection”. Since no interaction between “jugular side” and “time of collection” was observed, data were further analysed by a 3-way ANOVA (“jugular side”, “animal”, and “sampling period” as factors). A one-way ANOVA (“jugular side” as factor) was performed to identify animals that presented differences between jugular veins in the overall experiment as the mean of the four sampling periods. A one-way ANOVA (“night” and “day” as factor) was performed to study the overall differences between night and day melatonin concentrations.

To determine the repeatability of the melatonin concentrations a Pearson test was used between mean nocturnal melatonin concentrations for each sampling period and between melatonin concentrations from each jugular for each sampling period.

A Chi^2 test was performed to determine the potential existence of a non-uniform distribution in the cumulative percentage of animals that started their breeding season and those that ended it.

An analysis of variance was used to test the existence of a difference in absolute or relative melatonin concentrations between dates of onset and end of the reproductive activity. A correlation coefficient was calculated, using the Spearman test, between the dates of onset or end of reproductive activity or the duration of the ovulatory or the oestrous season and the nocturnal, diurnal, and relative plasma melatonin concentrations for each sampling period.

The relative difference between concentrations in the two jugular veins was quantified by a relative ratio equal to the difference between the left and right concentrations divided by the lowest one, and expressed as a percentage. A ratio of 100% means that the highest concentration is twice the lowest one.

3. Results

3.1. Effect of jugular vein and sampling period on melatonin concentrations

Diurnal melatonin concentrations were lower (5.1 ± 0.1 vs 5.2 ± 0.1 pg/mL, right and left jugular veins, respectively) than nocturnal melatonin concentrations (65.6 ± 2.7 vs 55.8 ± 2.3 pg/mL, right and left jugular veins, respectively) ($P<0.001$) in a similar way in each jugular vein.

The mean nocturnal and diurnal melatonin concentrations for each sampling period and for overall samples were not affected by the sampled jugular vein or the sampling period ($P>0.05$). However, there was a significant effect of the animal used in the study ($P<0.05$) in diurnal or nocturnal melatonin concentrations. In the same analysis, interaction between jugular vein and animal was observed in nocturnal melatonin concentrations ($P<0.001$). Figure 1 shows the distribution of animals with respect to the relative side-related difference in melatonin concentration. In 47.1% of the animals, the relative difference between jugular veins was greater than 100% (i.e. the highest concentration was at least twice the lowest one), and in 73.5% of the goats this difference was greater than 50%.

The one-way ANOVA revealed that in 18 goats (53% of the animals) melatonin concentrations were significantly different (at least $P<0.05$) between jugular veins. The relative difference between jugulars averaged 126.0 ± 14.7% for all individuals, and ranged from 0.1% to 1142.2%.

Table 1 shows the Pearson coefficients of correlation between the right and left jugular nocturnal melatonin concentrations and between periods. In general, it was observed that the highest significant ($P<0.01$) correlation coefficients were between the melatonin concentrations in the same jugular vein for each sampling period. However, the coefficients between melatonin concentrations in different jugular veins for the same sampling period or even for different sampling periods were lower, and in general non-significant.

3.2. Correlation between melatonin concentrations and onset or end of reproductive activity

Figure 2 shows the cumulative percentage of animals that started the breeding season or stopped it. The changes in the cumulative percentage for the onset of ovulatory activity occurred significantly faster than a uniform distribution ($P<0.001$). The distribution of the onset of oestrous activity and the end of the breeding season was not different from a uniform one. No differences in absolute or relative melatonin concentrations were observed between dates of first or last oestrous/ovulatory activity.

The Spearman coefficients of correlation between the first ovarian or oestrous activity, the last ovarian or oestrus activity, the duration of the ovulatory or the oestrous season and the nocturnal melatonin concentrations or the ratio nocturnal/diurnal melatonin concentration determined that all but one correlation coefficient did not significantly differ from zero; there was only one negative coefficient of correlation between the mean plasma melatonin concentrations during the night from both solstices and the first detected oestrus ($r=-0.36$, $P<0.05$).

4. Discussion.

The present study clearly shows for the first time in goats that, contrary to a widely-accepted assumption, the concentration of melatonin can differ considerably between the jugular veins of a given animal. In sheep, English et al. [20] found a possible difference in melatonin concentrations between right and left jugular veins only in 2 ewes. However, that result was biased because only two animals were used. In the present experiment, it has been observed that one animal can have higher melatonin concentrations on one jugular side than on the other, although no difference has been observed in the population. Consequently, it could have been that in the experiment of English et al. [20] two animals with differences between them were used. Therefore, to study the possible differences between jugular veins, it is necessary to use a high number and a random selection of animals in the experiment. Confirming this assumption, Zarazaga et al. [6] have recently demonstrated in a large number of animals that there is no absolute dominant side — in some individuals, higher concentrations were found on the right side, while in others they were on the left side, and in yet others, levels were similar on the two sides. Moreover, the authors demonstrated, using continuous collection of blood during the night that the higher melatonin concentration in one jugular relative to the other was stable throughout the night. In our case, we did not use fraction collection, but sampled the same animals in four different periods of the year; we found that a similar result was obtained, as the correlation coefficients for each side and for each sampling period were high.

The origin of this dissymmetry is unknown, but various possibilities can be postulated. The first would point to differences between the two jugular veins, in terms of diameter, blood flow, or connections with draining veins, which would lead to a different dilution of melatonin on each side. Another possibility is anatomical differences between animals, suggesting that the sagittal sinus distribution of blood carrying melatonin is not equal between the two transverse sinuses, which would explain the differences in concentration observed in the jugular veins.

The difference in melatonin concentrations between the two jugular veins has important practical implications for the assessment of melatonin secretion. Indeed, results may be different depending on whether melatonin is measured on the right side or the left. In a general population, mean melatonin concentrations are independent of the sampling side, as average concentrations are similar on the two sides. However, when the amplitude of the melatonin rhythm has to be assessed for each individual, the measurement on a single side will be misleading in many animals and will not provide a good index of the synthetic activity of the pineal gland of a given animal. Studies addressing relationships between the amplitude of the melatonin rhythm and physiological responses to melatonin may have been flawed by this failure of an accurate individual assessment of the amplitude. From a practical standpoint, a better assessment of melatonin production by the pineal gland can be obtained either by collecting blood simultaneously from both jugular veins and pooling the samples, or by collecting blood from the carotid artery, i.e. after the blood from the two jugulars has been mixed in the heart.

Analysis of melatonin concentrations per doe showed very high inter-individual variability but a high correlation coefficient between concentrations in the different sampling periods. This establishes that melatonin concentration is an individual characteristic of each animal, as was found in sheep [21], and — as in sheep [22] — could be under strong genetic control. Although it is clear that most of this inter-individual variability is intrinsic to the pineal gland, and may be related to its varying size between individuals [5,23,24], the unpredictable distribution of melatonin between the two jugular veins could explain in part this inter-individual variability, melatonin measurements being performed in a single jugular vein.

The absence of correlation between absolute or relative melatonin concentrations and the onset or the end of reproductive activity is consistent with recent results obtained in sheep [14]. That study, using a large number of animals, did not find any evidence indicating that these two parameters (date of onset/end of the breeding season and the mean plasma melatonin concentrations) are linked and that the variability in plasma melatonin concentrations is independent of the variability in the dates of onset or end of the breeding season. The present results confirm previous ones obtained in mares [25] suggesting that the pattern of secretion of melatonin plays a limited role in controlling the cessation of reproductive activity

These results contrast with those from previous experiments in sheep and in buffaloes. In one of those experiments, using melatonin-treated Ile-de-France ewes after long-days exposure, the interval between insertion of melatonin implants and onset of ovulatory activity was positively correlated with the relative melatonin concentrations [11]. In another, performed in non-implanted ewes of a reduced-seasonality breed (Rasa Aragonesa) maintained under natural photoperiod, a high correlation (r=0.84) was found between night/day ratio and date of the first oestrous activity [12]. Similarly, Italian buffaloes — non-seasonal animals — presented a low variation in melatonin concentrations between night and day [13]. Selection for fertility in autumn lambing affects patterns of melatonin during the dark phase, with lower melatonin plasma concentrations in ewes selected for higher spring fertility [26]. Finally, it was recently observed that after a buck effect, animals with lower levels of melatonin showed an onset of cyclic ovarian activity about 12 days after buck introduction, while another group with higher melatonin concentrations started 22 days later [27]. Thus, it is possible that animals with reduced seasonality have reduced variation of melatonin concentrations between night and day. However, this was not the case in the present study. This divergence with the results obtained in the former studies may be attributable to the difference in the number of animals used. However, the results obtained in the present study are reinforced by its examining not only oestrous activity by introduction of males but also ovarian activity by progesterone concentrations.

The major influence of melatonin on the reproductive axis is exerted on the pulsatile secretion of LHRH/LH, and this effect could by itself be sufficient to explain the regulation of seasonality [7,8]. One explanation for the absence of a relationship between variability in plasma melatonin concentrations and variability in dates of the breeding season may be found in the route taken by pineal melatonin to control LHRH/LH pulsatility. Melatonin synthesised in the pineal gland is secreted directly into the cerebrospinal fluid (CSF) of the III ventricle [28], from where it may diffuse to reach the premammillary hypothalamus, where melatonin receptors controlling the LH pulsatile secretion are located [29]. Accordingly, the rest of the pineal melatonin flowing into the general circulation via the vein of Galen would not be that which controls the central effects of melatonin. Therefore, it is possible that the main role of CSF melatonin is the control of seasonality of reproduction, whereas the role of melatonin of the peripheral circulation is to control other traits — such as moult and/or thermoregulation [3] — depending on the action of melatonin on peripheral rather than central receptors.

In conclusion, it has been proved that melatonin concentrations in Payoya goats are highly variable between jugular veins in the same individual but not in the general population, that melatonin concentration is an individual characteristic indicating a possible genetic control of this trait, and that absolute or relative amplitude of plasma melatonin concentration is not related to the seasonal breeding activity in Mediterranean goats. The role of melatonin amplitude in goats originating from higher latitudes require further study.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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Table 1: Correlation coefficient between the mean nocturnal melatonin concentrations from each jugular vein (right or left) in each sampling period (spring equinox, summer solstice, autumn equinox, and winter solstice) in entire Payoya goats.

	RIGHT SPRING	RIGHT SUMMER	RIGHT AUTUMN	RIGHT WINTER	LEFT SPRING	LEFT SUMMER	LEFT AUTUMN
RIGHT SUMMER	0.82**						
RIGHT AUTUMN	0.62**	0.60**					
RIGHT WINTER	0.73**	0.67**	0.67**				
LEFT SPRING	0.17	0.07	0.14	0.12			
LEFT SUMMER	0.45*	0.44*	0.23	0.40*	0.62**		
LEFT AUTUMN	0.23	0.30	0.23	-0.01	0.53**	0.52**	
LEFT WINTER	0.51**	0.35	0.09	0.17	0.64**	0.71**	0.57**

*: $P<0.05$

**: $P<0.01$

Figure legends

Fig. 1: Distribution of the mean relative difference for each goat in melatonin concentrations between the two jugular veins in entire Payoya goats. The relative difference is equal to the difference between left and right jugular veins divided by the lowest one, and expressed as a percentage. Solid bars indicate relative differences higher than 100% between left and right jugular veins, i.e. the concentration on the higher side was at least twice as high as on the opposite side.

Fig. 2: Distribution of the cumulative percentage of animals starting the breeding season (upper) (first ovulatory activity (solid bars) and first oestrous activity (open bars)) and stopping it (lower) (last ovulatory activity (solid bars) and last oestrous activity (open bars)).

Figure

Figure 1:

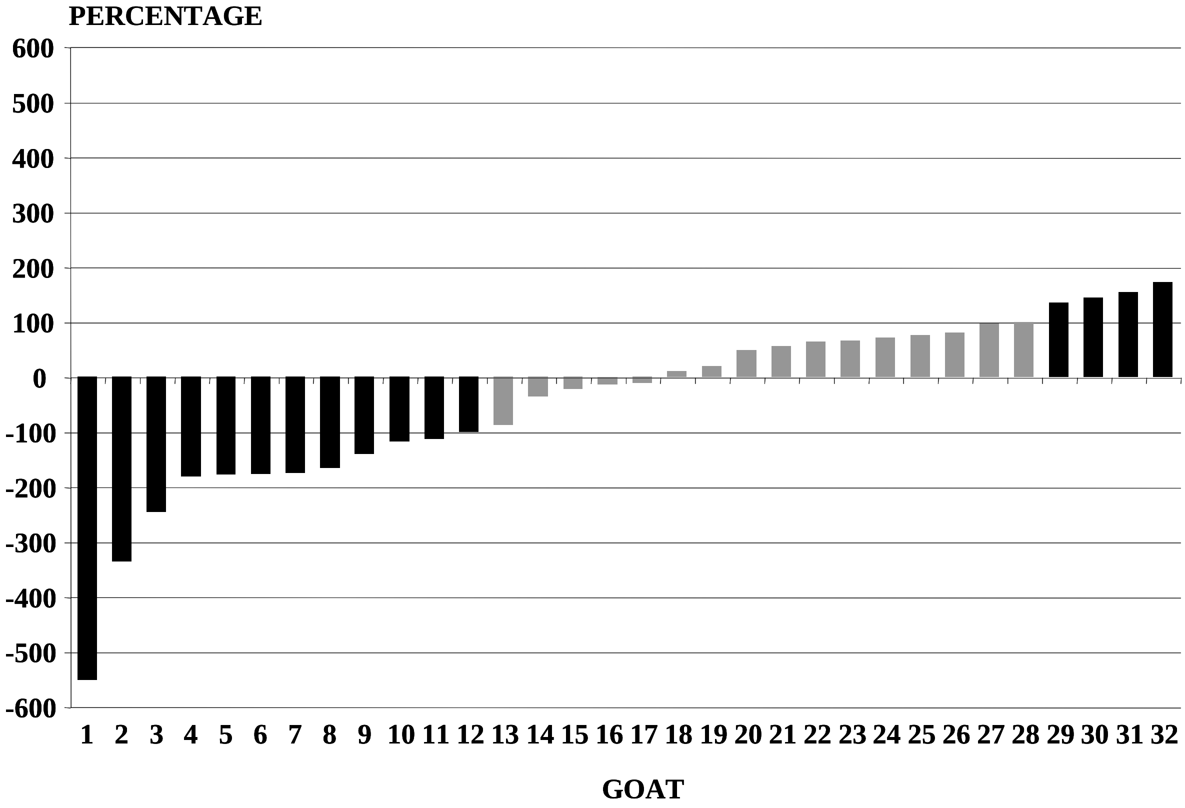
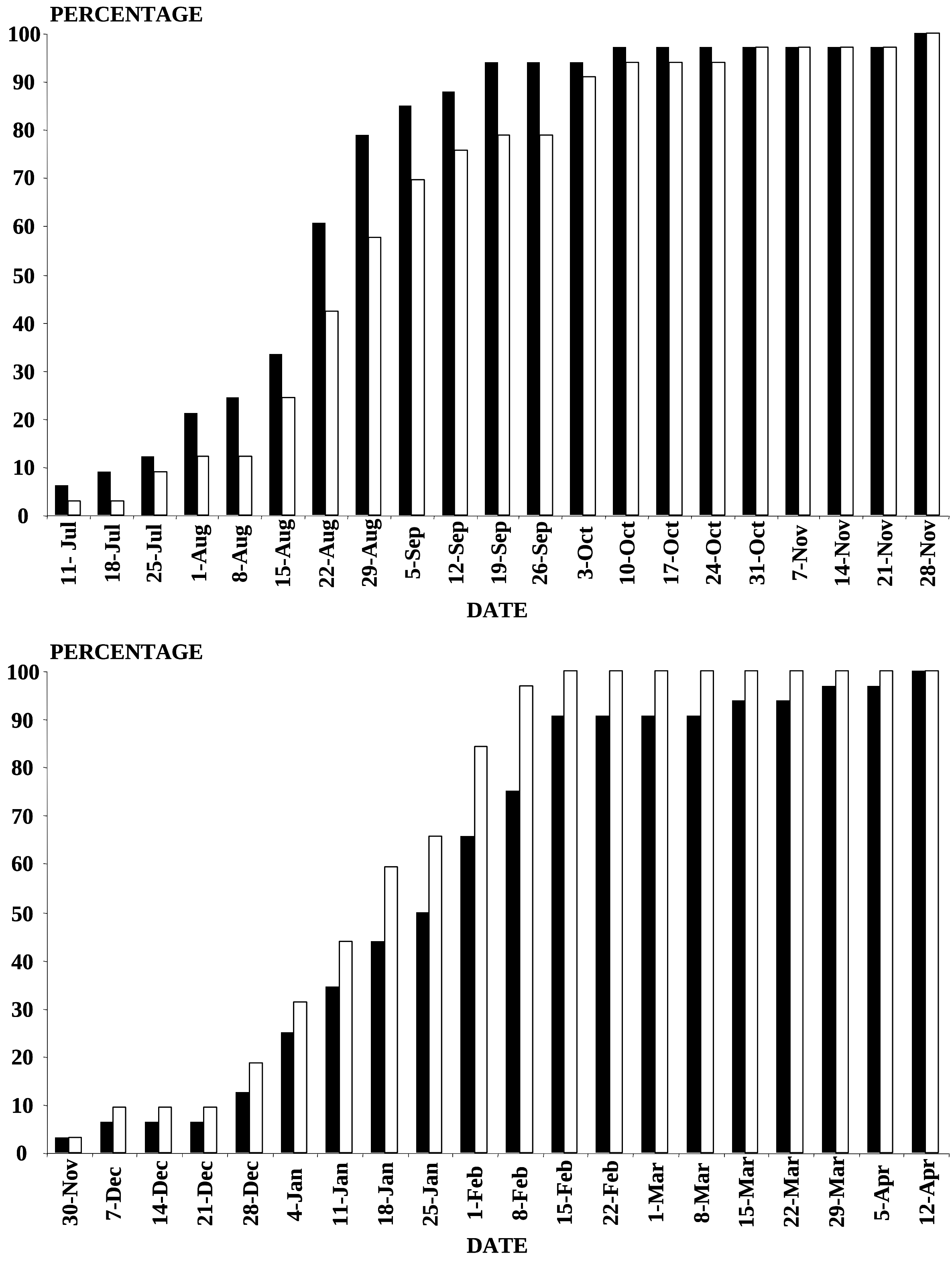


Figure 2: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