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Response to the sexually active buck effect in Beni Arouss goats primed with progestagens during the anoestrus and breeding seasons

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ABSTRACT

The response to buck effect (BE) was studied in Beni Arouss goats during anoestrus (AS) and breeding season (BS). Prior to AS, bucks were exposed to artificial long days during 75 days followed by a natural photoperiod. Goats of group 1 were treated for 11 days with 20 mg of flurogestone acetate (FGA) combined to 300 IU of eCG and 50 µg of cloprostenol injected 48 h prior to FGA-removal. Goats of group 2 and 3 were subjected to the same treatments, but eCG injection was replaced by a sexually active buck introduced 0 (group 2) or 48 hours (group 3) before FGA-removal. During the AS, 77% of goats induced by the BE showed an oestrus at [60–74] hr following FGA-removal for group 3 and [71–77] hr for group 2 ($p < .05$). In group 1, 77% of goats displayed an LH surge [24–40] hr after FGA-removal and 67% developed a luteal phase, but no LH surge or luteal response was detected in groups 2 and 3. During the BS, oestrus response reached 100% in goats synchronised with the BE ([22–68] hr in groups 2 and 3). In all groups, 84% of the goats displayed an LH surge at [30–70] hr post-treatment and 69% displayed luteal phase within 3–8 days post-treatment. After 11–15 days, the occurrence of ovulations followed by normal luteal phase was raised in all groups (91%). It was concluded that photostimulated bucks failed to induce and synchronise ovulation in goats previously treated with 20 mg of FGA and 50 µg of cloprostenol during anoestrus.

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HIGHLIGHTS





- The response to the buck effect was studied in Beni Arouss goats during anoestrus and breeding season.
- The use of sexually active bucks to induce and to synchronise oestrus and ovulation in goats treated with 20 mg of FGA and cloprostenol during the anoestrus season is not efficient in comparison with an hormonal treatment based on administration of FGA, eCG and cloprostenol.
- The same protocol appears as an adequate alternative for oestrus and ovulation synchronisation during the breeding season.

Introduction

In Beni Arouss North Moroccan goat, there is an alternation between a breeding season characterised by a succession of oestral cycles and the anoestrus season characterised by an anoestrus and anovulatory periods (From April to last June) (Chentouf et al. 2011). In males,

capacity for reproductive performance is maximal during the summer and autumn (El Kadili, Raes, et al. 2019a).

The increasing need for conserving this autochthonous genetic resource (FAO 2007) and promoting farm productivity and economy necessitate strategies to induce and synchronise reproductive functions

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during anoestrus and breeding periods. These strategies are also required to apply scheduled artificial inseminations (AI) as part of a breeding program.

Several protocols based on the use of progestagens to mimic a luteal phase, and eCG to stimulate ovarian follicular growth and ovulation are applied to goats. The most efficient one applied to Beni Arous goat consisted of administering progestagen by use of an intravaginal sponge (20 mg, FGA) for 11 days, followed by an injection of 300 IU eCG and 50 µg cloprostenol 2 days before sponge withdrawal (El Kadili et al. 2019b). Although, it is efficient at short term, repeating this treatment decreases fertility as a consequence of anti-eCG antibodies production (Roy et al. 1999; Drion et al. 2001; Sun et al. 2019). Moreover, routine production of eCG from pregnant mares raises important ethical issues in relation to animal welfare (Pellicer-Rubio et al. 2019). These limitations combined with the encouragement of farmers by the society to adopt safe practices by reducing or completely avoiding the use of exogenous hormones warrants new research aiming to develop alternative methods of oestrus and ovulation synchronisation in AI programs (Martin et al. 2004).

After an isolation period, the stimulation of females by sexually active males previously photo-stimulated with artificial light induces and synchronises ovulation in anovulatory goats (Delgadillo and Vélez 2010; Luna-Orozco et al. 2012). The phenomenon of sexual biostimulation is called 'the buck effect' and it is used in field conditions as non-pharmaceutical manipulation to advance reproductive activity in non-cycled females. After introduction of buck among the does, ovulation is detected around the third day of contact (Rekik et al. 2012; Delgadillo et al. 2017; Pellicer-Rubio et al. 2019). However, in most cases this first ovulation is followed by a short luteal phase (5–7 days), which decreases the conception rate. A normal second ovulation accompanied by oestrus behaviour occurs 7 to 9 days after buck introduction (Chemineau et al. 2006; Rekik et al. 2012; Pellicer-Rubio et al. 2016). To avoid short ovarian cycles induced by male effect, goats should be primed with progestagens or natural progesterone before the introduction of buck, thereby improving the fertility of the first ovulation (Chemineau et al. 2006; Pellicer-Rubio et al. 2007).

In small ruminants, the effectiveness of scheduled AI after classical or alternative treatments is determined by establishing the optimal interval between the end of treatment and insemination. Determining oestrus onset and preovulatory LH surge time after treatment is effective to establish the optimal time for insemination.

The aim of the present study was to assess in spring (seasonal anoestrus) and autumn (reproduction season) the efficiency of a FGA–PGF_{2α} treatment combined to a buck effect started 48 hours before or at FGA sponge removal on oestrus and ovulation in Beni Arous goats.

Material and methods

Animals and management

All animal procedures were approved by the Animal Ethics Committee of INRA. This study was conducted at the experimental station of INRA, Regional Centre of Tangier, located in the North of Morocco (latitude 35°44'N, longitude 5°54' O) during the anoestrus season and the breeding season.

In both study parts, goats were maintained in a semi-intensive system under natural photoperiod with access to pasture. Oat hay and concentrate feed mixture were distributed once a day according to the maintenance requirements (Jarrige 1988) with water and mineral salt available *ad libitum*.

Females were strictly separated from males (non-present on site) for at least 1 month before the start of the study.

Four bucks were used to stimulate females in this study (2 bucks per group). They were maintained indoors in individual pens in another barn isolated from the females (20 Km of distance) and fed oat hay and concentrate feed mixture distributed according to the recommended requirements of Jarrige (1988) with water available *ad libitum*.

Experimental design and treatments

The first part of the study was conducted during April (anoestrus season) and the second part was performed in November (reproduction period). Forty-three (anoestrus) and 45 (reproduction period) non-lactating Beni Arous goats aged between 3 and 6 years with a live weight of 29 ± 4 kg were used. All females had given birth at least once before entering the study and were randomly assigned to three treatment groups in function of age and body weight. At beginning of the protocol, absence (during anoestrus) or presence (during reproduction period) of a luteal phase was assessed by plasma progesterone 8 and 0 days before vaginal sponge placement. A vaginal sponge delivering 20 mg of fluorogestone acetate (FGA; Chronogest LC[®], Intervet, France) was placed during 11 days and 50 µg of cloprostenol (Estrumate[®], MSD animal Health, Morocco) was administered intramuscularly 48 hours before

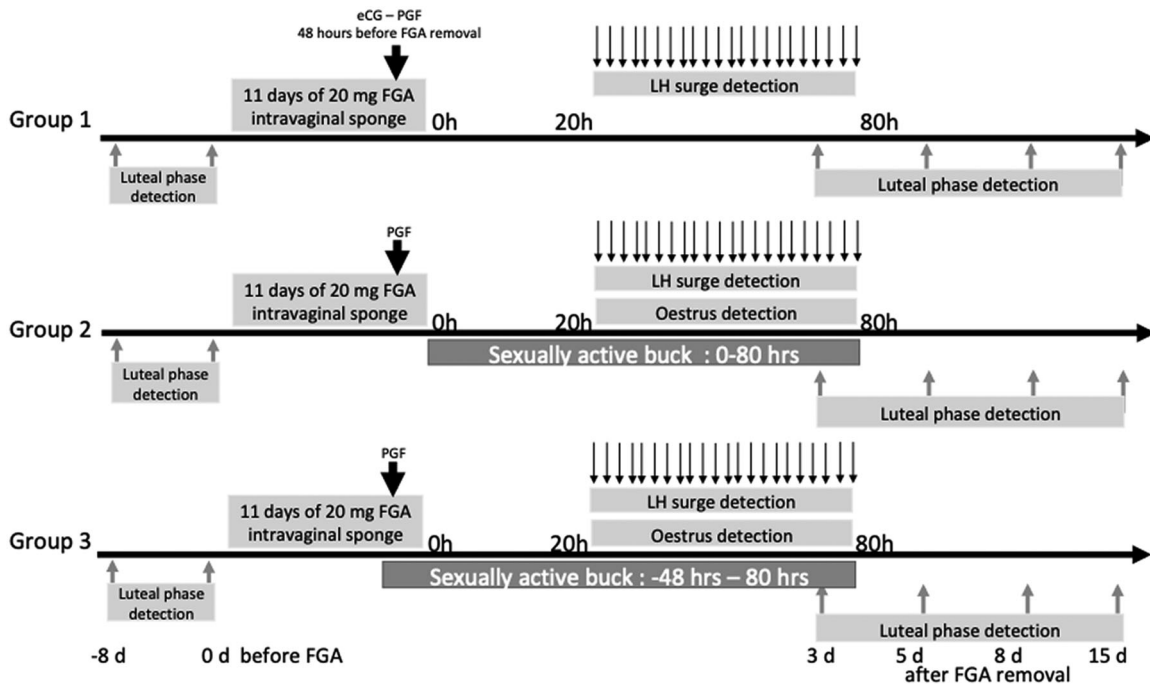


Figure 1. Experimental design. FGA intravaginal sponge: fluorogestone acetate impregnated intravaginal sponge ; eCG: equine chorionic gonadotropin; PGF: prostaglandin analogue (cloprostenol) ; LH: luteinizing hormone, hrs: hours; d: days.

sponge removal. In group 1, considered as control, 300 IU of eCG (Synchro-part, Ceva, Morocco) was also administered intramuscularly 48 hours before sponge removal. In group 2, eCG injection was replaced 48 hours before sponge removal by sexually active, but aproned bucks. In group 3, aproned bucks were used instead of eCG injection and were introduced at sponge removal. In order to prevent buck effect in group 1, goats were kept in complete isolation (>100 m, indoors) from the groups 2 and 3. FGA and cloprostenol treatments in groups 2 and 3 were timed in order to allow a simultaneous introduction of the bucks that were maintained in the groups until 80 hours after sponge removal. Oestrus behaviour was recorded between 20 and 80 hours after sponge removal in groups 2 and 3 where bucks were present. In all groups, blood samplings performed at 2 hour intervals between 20 and 80 hours after sponge removal aimed at assessing the preovulatory LH surge. Further samples were taken 3, 5, 8 and 15 days after sponge removal and aimed at detecting a luteal phase by dosing progesterone. The experimental design is shown in Figure 1.

Application of photoperiodic treatment to stimulate sexual activity in bucks during spring

The preparation of the bucks started before anoestrus season, on 14 November. They were submitted to a photoperiodic treatment of long days (16 h of light

and 8 h of darkness per day) from 14 November to 28 January. This treatment was provided using the light method in open barns and the intensity of the artificial light provided was at least 200 lux at the level of the eyes of the animals. Artificial light was regulated by an automatic clock and was provided from 06:00 to 09:00 h and from 18:00 to 22:00 in order to obtain a total of 16 h of light per day. On 29 January, the light treatment was stopped, and the bucks were exposed to natural day length variations until the end of the study (24 April). This treatment has previously been shown efficient to stimulate the male reproductive activity during the non-breeding season (Veliz et al. 2006; Chentouf and Bister 2011; Chasles et al. 2016; Delgadillo et al. 2017).

Luteal phase detection by plasma progesterone measurement

Blood samples were collected from all goats via the jugular vein using 9 mL heparinised vacutainer tubes. To establish the reproductive status of goats, blood samples were collected 8 days before sponge insertion, the day of sponge insertion and 3, 8, 11 and 15 days after sponge removal. Plasma was separated by centrifugation at 800 g for 20 min, transferred into 1.5 mL microcentrifuge tubes and stored at -20°C until the assay. Progesterone concentrations were measured in duplicate samples using a commercial ELISA kit (abia

Progesterone DK.039.01.3, AB Diagnostic Systems GmbH, Germany). The detection limit of the assay was 0.1 ng/mL, and the intra- and interassay coefficients of variation were 3% and 8%, respectively. Goats were considered to be in luteal phase when their plasma progesterone concentration was higher than 2 ng/mL at two successive blood samplings. They were considered to be in anoestrus if the samples performed at -8 and 0 days before FGA treatment revealed progesterone concentrations below 2 ng/mL.

Onset of oestrus

In group 2 and group 3, the oestrus behaviour was checked every hour from 20 to 80 h after sponge removal by the same bucks used in female's stimulation already fitted with a plastic apron and a colour harness. The goats were considered to be in oestrus only if they stood while be mounted by the bucks and showed a colour marks.

Pre-ovulatory LH surge

Plasma LH concentration was measured every two hours from 20 to 80 h after sponge removal. The collected blood was treated as previously described. Concentrations of plasma LH were determined in duplicate samples using commercial ELISA kit (LH Detect for caprines, ReproPharmVet, INRA, France). The detection limit of the assay was 0.1 ng/mL, and the mean intra-assay and inter-assay coefficient of variation were respectively 3% and 11%. Pre-ovulatory LH surge was determined as the time of the peak LH concentration with at least, a fivefold amount of the basal LH concentration.

Statistical analysis

Based on plasma progesterone analysis performed on samples collected prior to hormonal treatment (-8 and 0 days before FGA treatment), goats displaying ovarian activity in spring ($n=2$) and goats displaying

absence of ovarian activity in autumn ($n=0$) were excluded from the dataset. The number and proportion of animals within each season and each group displaying signs of oestrus, a pre-ovulatory LH surge and onset of a luteal phase after 3 to 8 and after 11 to 15 days after FGA sponge removal was calculated. Peak LH concentration detected during surge, and the interval between FGA sponge removal and onset of oestrus and LH surge were calculated for each animal and expressed as medians with their minimum and maximum in function of season and group.

The normal distribution of the data was assessed using the Shapiro-Wilk test. The Kruskal-Wallis and Wilcoxon sum rank tests were used to analyse non-parametric data with treatment (group) as main effect. Frequency data were assessed by the Fisher's exact test. The significance level was set at ($p < .05$) in all tests. The R statistical software (version 3.5.1) was used for data analyses.

Results

No sponge losses were recorded during both parts of the study. Progesterone measurements performed before sponge insertion revealed that all goats were in anoestrus during the first part of the study and representing an ovulatory activity during the second part.

During the anoestrus season in spring, most of the goats of groups 2 and 3 stimulated by the male effect showed a similar oestrus response rate (77%) at an interval ranging from 60 to 77 hours following sponge removal (Table 1 and Figure 2(A)). Oestrus occurred significantly earlier in goats of group 3 (68 [60-74] hours) than in goats of group 2 (74 [71-77] hours; $p < .05$). In group 1, oestrus remained voluntarily non-detected and 77% of goats displayed pre-ovulatory LH surge (peak LH concentration of 25 [11-42] ng/mL) at an interval ranging from 24 to 40 hours after sponge removal. However, none of the goats induced by the male effect showed a pre-ovulatory LH surge (Table 1, Figure 2(B and C)). No luteal phase occurred within 3 to 8 days or 11 to 15 days after sponge removal in

Table 1. Treatment effect on oestrus induction, pre-ovulatory LH surge and onset of luteal phase within 8 and 15 days post FGA removal. Data are shown as absolute numbers and proportions of responding goats.

Season	Group	FGA	PGF2alpha	eCG	Buck introduction	Oestrus detected	LH surge	Luteal phase after 3-8 days	Luteal phase after 11-15 days
Spring ($n=43$)	1 ($n=13$)	20 mg	50 µg	300 IU	-	NA	77%	67%	67%
	2 ($n=15$)				0 hrs	87%	0%*	0%	0%
	3 ($n=15$)				to 48 hrs	67%	0%*	0%	0%
Autumn ($n=45$)	1 ($n=15$)	20 mg	50 µg	300 IU	-	NA	73%	73%	93%
	2 ($n=15$)				0 hrs	100%	87%	67%	87%
	3 ($n=15$)				to 48 hrs	100%	93%	67%	93%

FGA: fluorgestone acetate; eCG: equine chorionic gonadotropin; PGF2alpha, prostaglandin F2alpha. NA: Oestrus was not assessed as no bucks were introduced in group 1. *: No LH surge was detected between 20 and 80 hours after FGA sponge removal.

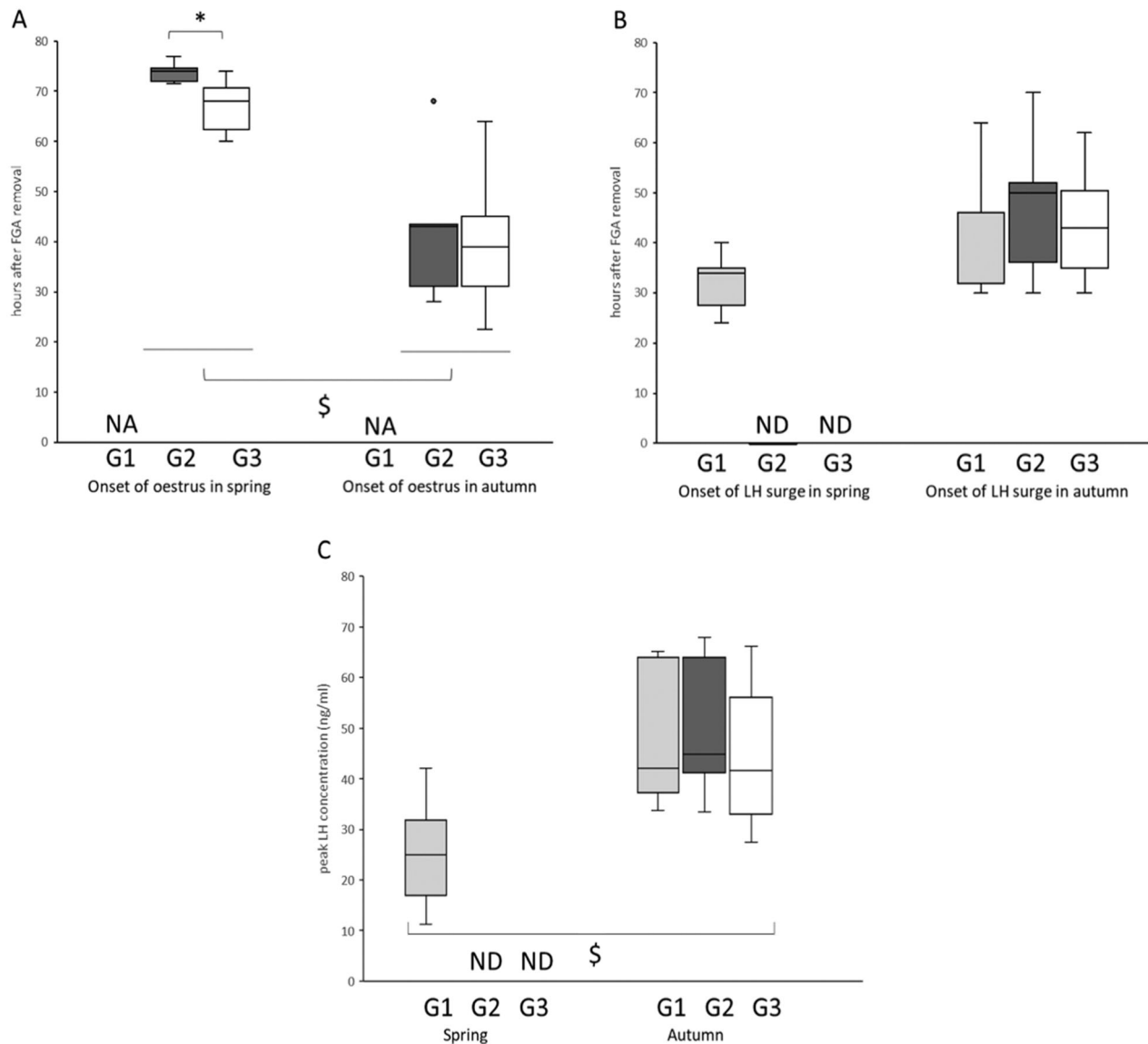


Figure 2. Onset of oestrus (A) in spring and autumn and time point of LH surge recorded in spring and autumn (B) and peak LH concentration at surge recorded in spring and autumn (C) in Beni Arouss goats' groups G1, G2 and G3. NA: Oestrus was not assessed in groups G1. ND: no LH surge was detected between 20 and 80 hours after FGA sponge removal in G2 and G3 investigated in spring. Results are expressed in hours (median with minim and maximum). * indicates a significant group-related difference ($p < .05$). \$ indicates a season-related difference ($p < .05$).

groups 2 and 3, whereas 67% of goats in group 1 developed an active *corpus luteum*.

In group 1, one goat died suddenly for unknown reasons after the LH detection period (i.e. 80 hours after sponge removal). Moreover, another goat of group 1 displayed a pre-ovulatory LH surge but did not ovulate nor developed *corpus luteum* within 15 days following sponge removal.

For the treatments based on male exposure during the sexual season in autumn, oestrus response rate in groups 2 and 3 was of 100% and occurred between 22 and 68 hours after FGA sponge removal, which was

significantly earlier than in spring (Table 1, Figure 2(A)). Animals of all 3 groups displayed a pre-ovulatory LH surge (84% with a peak LH concentration of 42 [27–68] ng/mL) at an interval ranging from 30 to 70 hours following sponge removal (Figure 2(B and C)). The percentage of females displaying a luteal phase within 3 and 8 days from sponge removal did not differ among treatment groups ($p > .05$), with an overall percentage of 69% (Table 1). After 11 to 15 days, the occurrence of ovulation followed by a normal luteal phase was raised in all groups and reached 91% without significant differences between groups ($p > 0.05$).

Discussion

This study investigates, for the first time, the efficiency of the buck effect in Beni Arouss goats. If the so-called 'classical treatment' based on FGA, eCG and cloprostenol was efficient in spring during anoestrus and in autumn during the reproduction period and confirmed earlier studies (El Kadili et al. 2019b), the introduction of a buck 48 before or at FGA sponge removal instead a eCG treatment failed to induce an appropriate ovarian response during anoestrus. In autumn however, both tested treatments were efficient.

Although the present study does allow providing answers regarding the efficiency of the buck effect in Beni Arouss goats, several limitations should be pointed out. The animals investigated in group 1 were not exposed to a sexually active buck in order to compare the impact of eCG treatment with the buck effect. As a consequence, no data about onset of oestrus were available. The use a fourth group receiving no eCG and PGF_{2α} and a fifth group receiving the 'classical protocol' plus a buck effect would have been interesting in order to test the efficacy of buck effect in both seasons and especially in reproduction season in which PGF_{2α} is sufficient to synchronise the reproductive activity. Another unexpected weakness was the delayed onset of oestrus in spring: even it is unlikely that an appropriate LH surge occurred in animals of group 2 and 3 (no luteal phase occurred within 15 days), the time frame of blood collection should have been increased above 80 hours post sponge removal in order to assess whether LH increased or not. It would furthermore have been interesting to assess plasma oestrogen levels in these goats: oestrus signs occurred, but lately, without leading to ovulation. Characterising the follicular response would be helpful if further studies testing the buck effect are performed.

During both seasons, the response to the standard protocol did not differ with an overall pre-ovulatory LH surge response of 75% and a median interval to LH surge of 34 hours (range 24 to 64 hours) following sponge removal. In general, the response was relatively similar to what is reported in our previous study (El Kadili et al. 2019b) and in other breeds such as Blanca Andaluza (Zarazaga et al. 2014), local population in Tunisia (Rekik et al. 2014) and Alpine and Saanen breeds (Leboeuf et al. 2003). In the last ones, insemination was performed 43 ± 1 h after sponge removal and resulting good fertility in goats (kidding rate of 70%). In fact, considering pre-ovulatory LH surge occurred around 31 hours, and taking into account the relatively constant 22 hours between the

occurrence of pre-ovulatory LH surge and ovulation, the insemination is performed 12 hours after LH surge. On the basis of our results, since the LH surge is more recorded around 34 hours after sponge removal, the goats could be inseminated at 46 hours.

Results of plasma LH concentration measured in spring revealed that the same goat of group 1 displaying a pre-ovulatory LH surge but did not ovulate, had a small level of LH surge (peak LH concentration of 12.9 ng/mL), which affirms that an inadequate release of LH because of a deficiency in GnRH synthesis and secretion, conducted to an ovulation failure in females (Noakes et al. 2009).

During the breeding season, three goats did not display a pre-ovulatory LH within 80 hours after sponge removal but were recorded as having ovulated within 11 to 15 days after sponge removal, which indicates a late occurrence of LH surge.

Curiously, during the anoestrus season, goats induced by the buck effect showed only an oestrus response (77%) but which occurred later at an interval of 60 to 77 hours after sponge removal. When compared to results obtained in goats treated with the standard protocol in our previous study (El Kadili et al. 2019b), in which oestrus onset was monitored (27 [26–46] hours), a delay in oestrus onset was detected in groups stimulated by the buck effect. This could be attributed to the effect of progestagen priming. The role of progestagen is to increase the time for follicular maturation and delay the preovulatory LH surge in order to prevent the appearance of corpus luteum with short lifespan induced by the use of buck effect alone in anovulatory females (Chemineau 1985; Lassoued et al. 1995; Chemineau et al. 2006). Moreover, during the anoestrus season, negative feedback of oestradiol is relatively greater comparing with what occurs during reproductive season, therefore, the frequency of LH pulse release is reduced, resulting lack of ovulation occurrence (Chemineau et al. 1988). In this study, the absence of ovulation even after 15 days from sponge withdrawal let us presume either no LH surge was induced or goats' responses started lately, with an LH surge occurring after the 80 hours of blood sampling, and that the LH surge was not followed by ovulation. These results could be related to the low concentration of progestogen added in the sponge, which can be associated with abnormalities in follicle development, ovulation and luteal function (Viñoles et al. 1999; 2001). Also, by comparing peak LH levels in goats treated with the classical protocol during anoestrus and sexual seasons, we clearly identify that LH level was lower during seasonal anoestrus

(peak LH concentration of 25 vs 42 ng/mL). This result confirms that using a higher dose of progestagen could advantage suppressing the great negative feedback of oestradiol induced in non-breeding season. Previous findings reported by Chentouf and Bister (2011) showed a pre-ovulatory LH surge response of 86% detected at an interval of 55 hours (± 13.5) after buck introduction in goats from the same breed when stimulation is performed by photoperiod treated bucks and primed by a progestagen (45 mg of FGA) in April. Likewise, in our study bucks were already submitted to a photoperiodic treatment to improve the response of goats to the biostimulation. However, the goats were treated only with a dose of 20 mg FGA, low dose that may influence the efficacy of response. Furthermore, our results disagreed with those described in the studies of Pellicer-Rubio et al. (2007, 2008) in dairy goats where the photoperiodic treatment involved the bucks and goats, during deep anoestrus and the progestational treatment consisted on intravaginal sponges impregnated with 45 mg of FGA. An overall LH surge response of 94% was obtained in the first study (in 2007) with an interval buck introduction to LH surge of 54.3 hours (± 15.4) and an onset of oestrus monitored in 85% of goats 24 to 72 hours after joining. Indeed, more than 90% of goats ovulated within 15 days after buck introduction. Relatively similar results were reported, likewise, in the second study (in 2008) where authors were also verified that cervical insemination 52 h and 70 or 75 h after buck introduction and sponge removal results in a higher kidding rate (74%) similarly to a classical protocol (72%). Not far from Northern of Morocco (under similar latitude), the use of male effect in combination with an intramuscular injection of 25 mg of progesterone was relevant in Murciano-Granadina goats by concentrating oestrus and ovulation at 3–4 days after buck introduction in 87% of anovulatory goats (Diaz Delfa et al. 2002). Also, by subjecting the same breed to IMA-PRO2[®] protocol, Lopez-Sebastian et al. (2007) reported a high degree of oestrus and ovulation synchronisation. Oestrus behaviour was observed in 87% of goats, 24–48 h after cloprostenol injection and pre-ovulatory LH surge was detected in 83% of goats, 33–48 h after the end of treatment.

According to treatment chosen, many factors could explain this variability of responses to buck effect. As reported by numerous studies, photoperiodic treatment of bucks and goats allow highly synchronous and fertile reproductive activity when male effect is performed in the middle of seasonal anoestrus (Chemineau et al. 1996; Walkden-Brown et al. 1999;

Flores et al. 2000; Delgadillo et al. 2002; Pellicer-Rubio et al. 2007; Zarazaga et al. 2019) and especially in deep anoestrus breeds such as Alpine and Saanen, where photoperiod treatment of goats ensure and optimise synchronicity and fertility of ovulatory activity induced by the buck effect (Chemineau et al. 1986; Pellicer-Rubio et al. 2007). In addition, variations in female responsiveness, quality of stimulation provided by the male, prior isolation, nutritional status, exposition of bucks to oestrous females prior the joining, the age and experience of the males and age of the goats may influence the induction of an ovulatory response by the buck effect (Walkden-Brown and Restall 1993; Perkins and Fitzgerald 1994; Flores et al. 2000; Thimonier et al. 2000; Ungerfeld et al. 2008).

In the current study, two more findings are interesting to mention in relation to the use of buck effect during anoestrus. Firstly, the onset of oestrus occurred earlier when bucks were introduced 48 h prior to sponge removal (Figure 2(A)), which was in line with results obtained when the injection of eCG was performed 48 h prior to sponge removal leading to an advanced follicular growth and consequent LH surge (Ritar et al. 1984). Secondly, as shown by goats of group 2, the introduction of bucks at sponge removal seems to synchronise more efficiently the onset of oestrus in comparison with buck introduction two days before sponge removal. All females showed their oestrus within a time frame of 6 hours, possibly as a result of well synchronised endogenous rhythm of FSH secretion.

During the sexual season, where the use of the 'classical protocol' remained in line with a previous study (El Kadili et al. 2019b) our results clearly indicate that the male effect protocol may be an adequate alternative for oestrus and ovulation induction and synchronisation in Beni Arouss goats. The time of buck introduction did not influence oestrus response (100%) or time to the onset of oestrus following sponge removal, with an interval ranging from 22 to 68 hours. Moreover, most of the goats responded to the treatments by displaying pre-ovulatory LH surge (from 30 to 70 hours) and ovulation, without significant differences in the timing of the occurrence. Based on our results, as the LH surge is more recorded between 30 and 52 hours after sponge removal (only 4 goats were outside this range) and considering 22 hours between the occurrence of the pre-ovulatory LH surge and ovulation, goats could be inseminated at 52 h after sponge removal during the breeding season.

Conclusion

This study showed that the use of a sexually active buck aiming to induce and to synchronise oestrus and ovulation in the Beni Arouss goats treated with 20 mg of FGA and cloprostenol during the anoestrus season is not efficient in comparison with an hormonal treatment based on administration of FGA, eCG and cloprostenol. However, the use of the buck effect in cyclic goats primed with progestagen treatment and intramuscularly injected with cloprostenol 48 h prior to sponge removal appears as a very promising alternative method for synchronisation of oestrus and ovulation in Beni Arouss goats. Although the synchronicity of the response to classical protocol and buck effect seems to be compatible with insemination at 52 h after sponge removal, the quality of ovulation and other factors could compromise the fertility after AI. The tested protocols need to be validated on a large number of animals and under field conditions.

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Ethical approval

All animal procedures were approved by the Animal Ethics Committee of INRA. This study was conducted at the experimental station of INRA, Regional Centre of Tangier, located in the North of Morocco (latitude 35°44'N, longitude 5°54' O) during the anoestrus season and the breeding season.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

The data that support the findings of this study are available on request from the corresponding author, [S.E]. The data are not publicly available due to their containing information that could compromise the privacy of research participants.

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