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Recent advances on synchronization of ovulation in goats, out of season, for a more sustainable production

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ABSTRACT

Goats show marked progressive seasonal reproduction at latitude > 25 ° and reproductive management should be adapted to market demands. The present review aimed to discuss the synchronization of ovulation for timed artificial insemination concerning new insights regarding a clean, green and ethical meat and milk production. Today, the induction of ovulation during breeding season or transitions periods is mainly based on progestagens/progesterone (P4) devices intravaginally inserted on females, at least during 11 days, plus equine chorionic gonadotropin (eCG) and prostaglandin F2 alfa administration. In last years a reduction to 20 mg of fluorogestone acetate was made and the successful reutilization of devices containing 0.3 g of P4 indicates a possible reduction of their levels. Shortening the period of exogenous progestagens/P4 priming (5 to 7 days) is critical for a rational use of hormones. Moreover, the eCG exchange by socio-sexual cues (male effect) seems to see a great advance, even if a previous photoperiod treatment, or equivalent method, being necessary in high latitudes. Research trends on these subjects are expected in future using different goats breeds in distinct regions of world.

1. Introduction

Goats, as well ewes, are spontaneously ovulating and commonly considered as seasonally polyestrous animals under temperate climatic conditions[1]. The photoperiod is one of the major factors that influence the reproductive activity in small ruminants[2,3]. Gradually, from subtropical regions to higher latitudes, most of local breeds show successive alternated breeding and non-breeding (anoestrous) seasons. This particularity have a great impact on reproductive and production management of flocks[1] and can imply different approaches between regions from different latitudes, breeds and seasons according meat and milk market demands during whole year.

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The artificial insemination is a major vehicle for genetic improvement of animal breeds and a reproductive management tool for farmers[4]. Females are normally inseminated following the hormonal synchronization of ovulation in flocks[5,6]. In the last decades, synchronization of ovulation protocols, out of season, are commonly based on controlled internal drug release (CIDR) or intravaginal polyurethane sponges impregnated with progesterone (P4), or their synthetic analogues (progestogens) mainly medroxyprogesterone, melengestrol and fluorogestone acetate forms, plus equine chorionic gonadotropin (eCG) and prostaglandin F2 alfa (PGF2) or even estrogenic pharmacologic active substances[6−12]. These protocols are dependent of country availability of licensed hormones [12,13].

The kidding rate can reach 65% after timed artificial insemination with frozen straws (100 × 10^6 spermatozoids/0.2 mL) in goats presenting estrus following a 11-day progestagen priming + eCG + PGF2 protocol and inseminated 43 to 46 hours after sponge withdrawal[14,15]. This fertility rate can be obtained during anoestrous season in regions with high latitude and mainly on

intensive dairy herds. Moreover, the possibility to use of sex-sorted spermatozoa in goats was also recently reported[16]. However, other worldwide extensive and semi-extensive production systems, concerning milk and/or meat products[17], also can profit with these reproductive tools, which should be adapted to the different local realities.

Today, the redesigning animal production systems for sustainable agriculture with a lower environmental impact and the adaptation to new hazards, such as the global climate changes, are significant challenges[18]. Animal welfare focused in reproductive management, should be also improved[19].

The use of hormones in animal production was strictly regulated, from last two decades, in several countries. For example, in European Union (directives 96/22/EC, 2003/74/EC and 2008/97/EC) the use of oestradiol 17 β (E2) in food-producing animals was banned and P4 utilization was limited, reducing hormones residues on food chain and environment, with potential benefices for public health. Consequently, a decrease from 45 mg to 20 mg of fluorogestone acetate in each sponge was approved, without apparent negative impact on goat fertility[20].

Due to the advent of the ultrasonography and molecular endocrinology as tools, animal welfare improvement and fertility increment of flocks, minimizing economic expanses, several researches were focused to shorten the duration period of intravaginal progestagen/ P4 device exposition from 11 or more days to 5-7 days in females[21], reutilize the intravaginal devices[22,23] or reduce the oxidative stress due to the device contact with the vaginal mucosa[24]. However, recently, a great attention was done to knowledge concerning the natural stimulations of ovulation, especially socio-sexual cues such as the male effect t[25] and even the female effect[26].

In the present paper, we discussed more significant recent advances concerning the synchronization of ovulation with potential impact on reproduction management systems, during the anoestrous season, at the specific goat reproduction contexts. The ultimate purpose was demonstrate that the lucid use of P4 or progestogens and male effect as a tool can achieve good practices of reproductive management in goats, compatible with a sustainable production.

2. Reproductive seasonality and anoestrous season

Goats and ewes are species presenting a reproductive seasonality, mainly according genotypes[27,28] and photoperiod stimulation[3,27,28]. Most of breeds originated from Latitude > 35° North or South and someone's in subtropical region, located between Latitude 35° and 25°, show a breeding season[15]. Toward to tropical regions, the reproductive seasonally of local small ruminants tends to disappear[29,30] and other factors, such as nutrition and environmental thermic stress (or other stressors), take place[31]. However, Delgadillo *et al*[25] recently observed that the continuous presence of sexually active bucks can prevent the display of seasonal anestrus of goats in Mexico (L 26° N). According these researchers, further studies are need in order to clarify the degree of the photoperiodic influence and other non-photoperiodic environmental factors, especially socio-sexual cues, on seasonality of goats.

At latitude > 45° N (temperate and polar regions), the onset of the breeding and non-breeding seasons of local breeds occur at the end of January/February/early Mars and late August/ September/early October presenting a transition period between seasons[1,32]. From Mars to September all goats remain without ovulatory activity[27]. In hemisphere south, anoestrous season occurs between October and January, like the reported by Rivera *et al*[33] in Argentina (L 30° S).

The annual patterns of reproduction activity is related with spring and winter solicits causing a progressive variation of daily light/dark duration. Light signals were detected by retina and processed by suprachiasmatic nucleus; signals arrives by sympathetic neurons via to pineal gland which produces melatonin, a key hormone, during short days/ darkness periods[35-37]. A neuronal network mediated by neurotransmission (dopamine, serotonin and other amino acids) is stimulated by melatonin in order to modulate the hypothalamic secretion of gonadotropin-releasing hormone (GnRH)[37]. There are several evidences that these photoperiod variations entraining an endogenous circannual rhythm, and the end and onset of breeding season were due to refractoriness to short and long days, respectively, entraining a circannual endogenous rhyming[38-40]. Is necessary approximately 40 days for the (re)stimulation of luteinizing hormone (LH) pulse activity by melatonin[41], but can reach approximately 66 days according breeds[42].

Chemineau *et al*[41] observed an increase of frequency and amplitude of LH secretion toward the breeding season, in Saanen goats, but the low plasmatic E2 levels remained constants suggesting a decrease of hypothalamic/pituitary sensibility to their inhibitory effects. The GnRH secretory neurons represents the output of the neural network responding to homeostatic and environmental stimulus which regulate the pituitary LH and follicle-stimulating hormone (FSH) secretion and both P4 and E2 hormones are also related with this system[43].

Above Latitude < 45° N, the anoestrous intensity, i.e. the degree of hypothalamic–pituitary-gonadal axis inhibition, indirectly measured during the non-breeding season according the percentage of females presenting spontaneous ovulations, gradually decrease[44]. The percentage of spontaneous ovulations were very well characterized in Blanca Andaluza goats by Gallego-Calvo *et al*[45] in Spain (L 37° N; Figure 1) and contrast with the 0% of spontaneous ovulations observed in France[27]. The anoestrous season is shortened, approximately from February/Mars to August like the reported in goats and ewes by some researchers[46,47], but a variable percentage of goats can ovulate before (June and July)[47], including during whole non-breeding season[45].

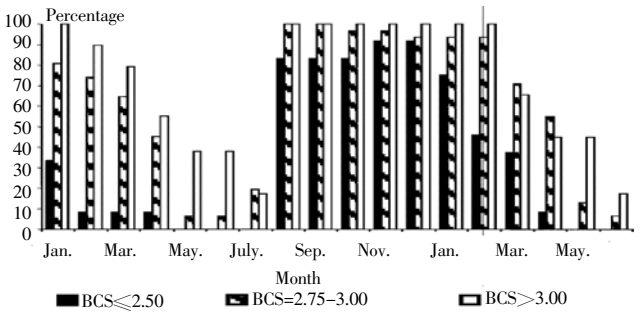

Figure 1. Monthly percentage of does showing ovulation (measured by progesterone concentration) depending on their body condition score.

The body condition score (BCS) was based on a 1 to 5 scale (1 = emaciated and 5 = extremely fat). A considerable percentage of goats presented ovulations in non-breeding season. Seven females showed regular cyclic ovulations over the entire experimental period[45].

The anoestrous intensity have practical implications in order to induce ovulation during the deep anestrus. For example, a photoperiodic treatment (16 h light and 8 h darkness during December to April) was necessary for the improvement of the females response to the male effect in France (L 46° N)[48], or lower dose of eCG can be used in local breeds of regions with lower latitude than 45°[49-51].

Breeds originated from a high latitude maintain the seasonality under a tropical photoperiod treatment (11-13 hour of light per day), but the ovulatory pattern can be influenced like the observed by Chemineau *et al*[27] in Alpine goats at the final phase of a 3 years study. Inversely, non-seasonal creole goats, from Guadalupe, presented ovulatory inactivity when subjected to a temperate photoperiod treatment (8-16 hour of light per day)[2].

All of these evidences indicates, that different reproductive management of goats, out of season, are necessary according animal breeds and geographic localization.

The anoestrous season is characterized by anestrus and anovulatory activities, but a follicular wave-like (modified) pattern on ovaries persist, in absence of corpora lutea[52]. Following the nonbreeding season, throughout the transition period to breeding season, a great percentages of females shows silence ovulations (mainly in ewes) or 5 -11 days short oestrus cycles (mainly in goats) due to prematurely regressing corpora lutea[53]. The occurrence of short oestrus cycles in goats could be due to an inadequate luteotropic support after ovulation, when LH pulses are essential for CL or premature activation of the luteolytic mechanism[11,54,55].

During the breeding season, the length of oestrus cycle is, in average, 21 days[1] until conception or the end of season, when another similar transition period occurs. A new wave emerge each 5-7 days and the last follicular wave of oestrus cycle origin one or more ovulations accompanied by estrus behavior[56,57].

3. Improving the progestagen + eCG + PGF2α protocols

3.1. Conventional protocols

Today, the principal hormonal protocols used in goats industry are based on devices contained progestogens/P4. Intravaginal polyurethane sponges or CIRD, an inert silicone elastomer, are impregnated with 20-40 mg of fluorogestone acetate, 50-60 mg of medroxyprogesterone or 0.3 g of P4[12]. These devices are inserted intravaginally, in elective goats, for 11 days or more (up to 21 days).

Studies about P4 were firstly reported for ewes[58] and subsequently adapted to goats[59-61]. Progesterone modulates the pituitary LH secretion, inducing a negative feedback, modifying the hypothalamic GnRH activity[62], followed by a pre-ovulatory LH surge after device withdrawal, if eCG is administered in order to improve the development of follicles, including the pre-ovulatory follicle(s), in anoestrous females. Contrarily to LH, P4 don't influence basal (pulsatile) and wave-like FSH secretion, at least in early oestrus cycle phase of goats[63]. The FSH is modulated by inhibin A and E2 produced mainly by large (dominant) follicle(s)[64].

At time device withdrawal, 24 or 48 h before[65], PGF2 or their synthetic analogues, and eCG are administered i.m. in order to promote the luteolysis of potential corpora lutea (if present in some goats) and the development of antral (preovulatory) follicle(s), respectively. The eCG have a primordial FSH effect and secondarily a LH effect, and acts directly at ovaries level. However, this glycoprotein can develop antibodies when two time successively administered with adverse effect on fertility of the same goat when treatment is repeated in next anoestrous season[5,66]. Their substitution by E2 was tested seems to showing poor results[49].

The eCG, normally administrated from 250 to 600 IU i.m. according breeds, latitude, milk yield (<3.5kg *vs*. 3.5 kg/day), parity of female, post-partum delay and season, is the key for the induction of ovulation in anestrus goats[4,19,67]. However, Leyva *et al*[68] observed that the progestagen priming increased the number of follicles stimulated by eCG and consequently the ovulation rate in anestrous ewes. Moreover, the progestagen priming can synchronize the ovulatory wave[11].

These devices impregnated with P4 or progestogens should be excreted the exogenous hormone below the residues limit in milk approved by official authorities. The P4 levels in milk, detected after device use (0.3 g of P4) were lower than that endogenously produced during diestrus or pregnancy[69,70]. However, according Lopez-Sebastian *et al*[71], a high residue concentration in milk can be observed in the first few days after the sponge insertion impregnated with 20 mg of fluorogestone acetate. This last aspect requires attention and more researches are necessary to determine if the fertility rate remain unaltered with a lower fluorogestone dose, even if a previous photoperiod (or melatonin implant) treatment is necessary.

Also, the successful reutilization of devices[22,23] suggest that the exogenous P4 concentration can also be reduced. However, new researches are need in order to evaluate their (null) impact on fertility rate and follicular dynamics, like the reported for fluorogestone

acetate[20,72].

These conventional protocols were developed following several studies concerning the pre-ovulatory events evaluation, such as the LH preovulatory peak and ovulatory follicle disappearance observed mainly by invasive laparoscopy[53] and the fertility rate results. Therefore, the follicular dynamics before ovulation was not sufficiently evaluated. With the advent of the ultrasonograhic tool, a noninvasive technique, and their association with molecular endocrinology represented an important advance. Several studies concerning the follicular and corpus luteum dynamics were also performed in goats [11,56]. Consequently, the ovarian morphologic dynamics can be more easily related with hormonal events[56,57], minimizing potential adverse stressors due to successive animal manipulations. A potential important impulse on this subject was done by Uruguayan researchers.

3.2. 5 to 7-days short progestogen priming protocols

Rubianes *et al*[73] observed that the plasmatic P4 levels remained higher (>5 ng/mL) for 3 or 4 days after a device insertion on anoestrous goats than those observed during the mid-late luteal of cyclic females, but decreased to subluteal levels (2 ng/mL) until the end of treatment (device withdrawn). It was suggested that the conventional 11-days progesterone priming induce low plasmatic P4 concentrations toward the end of treatment affecting LH secretion pattern and consequently follicular development (oocyte health and ovulation) and fertility[73-76].

The follicular status at the time of progestogen device insertion is also very important. Using cyclic ewes, Noël *et al*[77] observed that fluorogestone acetate accelerated the mechanisms of follicular growth, reducing the number of large follicles during luteal and increasing the atresia rate in luteal phase and was detrimental to both the number of large follicles and the ovulation rate during follicular phase. When sponges impregnated with medroxyprogesterone acetate were inserted on Day 6 (toward middle luteal phase), the time of the preovulatory LH surge and ovulation were delayed compared with ewes in which sponges were inserted on Days 0 and 12 of estrous cycle.

Viñoles and Rubianes[78] observed in ewes that the dominant follicles in the growing or plateau phase at the time of luteolysis became ovulatory. Inversely, if it was already in the regressing phase, the dominant follicle of wave 2 became the ovulatory.

Menchaca and Rubianes[79] observed that the device P4 insertion affected the lifespan of the largest follicle of wave 1 and advanced the emergence of wave 2 in early phase of the oestrus cycle. After several studies in cycling dairy goats submitted to short P4 priming (5-7 days), Rubianes and Menchaca[11] hypothesized their effect on follicular dynamics (Figure 2), claimed an unjustified long progestogen/P4 priming and suggested that this protocol short can be used successfully in both anoestrous and cycling goats.

Menchaca and Rubianes[49] reported the short P4 priming plus 200-300 IU of eCG and PGF2 use during breeding and anoestrous seasons in Uruguayan small dairy goat farmer (L 34° S). Globally, estrus behavior rate reaching 90 % and pregnancy rate reaching 60% after timed artificial insemination, without significant differences between seasons. In non-breeding season (April; L 40° N), the oestrus behavior rate (50%) and pregnancy rate (62.5%) after a 6-days short-term progestogen (20 mg of fluorgestone acetate) priming followed by male effect treatment (in substitution to eCG) and AI was also acceptable in Serrana goats[80].

So, the short-term progestogen/P4 priming (and reduction on device P4 concentration) seems to be a rational protocol and should be widely tested in different breeds and latitudes (Table 1) in both cyclic and anoestrous goats.

Table 1

Hormonal protocols tested in goats in anoestrous season or transition period.

World region	Breeds	Protocol	% estrus	Pregnancy rate (%)	Reference
France, L 46° N(1)	Alpine and Saanen goats	Fluorogestone acetate (20 mg) during 12 days + eCG (500 IU) + cloprostenol (50 μg) (i.m., 2 days before sponge withdrawal); TAI: 43±1 h	100% (98)	69.1% (67/97)	[20]
Uruguay, L34° S(1)	Alpine, Saanen and Anglo Nubian lactating goats	Medroxiprogesterone acetate (60 mg) during 5-6 days + eCG (250 IU) + Delprostenate (160μg) (i.m., at sponge withdrawal); TAI: 54 h	91.7% (154/168)	63.7% (107/168)	[49]
Spain, L 37° N (non-breeding season)	32 Blanca Andaluza goats	Fluorogestone acetate (20 mg) during 11 days + eCG (450 IU) ++ luprostiol (6 mg) (i.m., 2 days before sponge withdrawal); Not inseminated	92%	–	[51]
Italy, L41° N transition period (June-July to September)	Indigenous dairy goats	Fluorogestone acetate (45 mg) + during 5 days + eCG (300 IU) + cloprostenol (50 μg) (i.m., at sponge withdrawal); Natural mating	78.3% (18/23)	60.9% (14/23)	[50]
		Fluorogestone acetate (45 mg) + during 5 days + eCG (300 IU, i.m., at sponge withdrawal) + cloprostenol (50 μg, i.m., at sponge insertion); Natural mating	86.4% (19/22)	63.4% (14/22)	
		GnRH (100 μg, i.m. at D0) + eCG (300 IU) + cloprostenol (50 μg) both at D5(2); Natural mating	58.3% (14/24)	41.7% (10/24)	

(1)Two periods (breeding and non-breeding seasons) were used. However, no significant differences were observed between seasons.
(2)Progestagens or P4 were not used. Authors do not clarified the percentage of anoestrous goats before treatment. Additional support is need in order to evaluate in GnRH exchange follicular dynamics during late anoestrous season in goats.
eCG: Equine chorionic gonadotropin; TAI: timed artificial insemination; GnRH: Gonadotropin-releasing hormone.

Finally, one of the inconvenient of intravaginal devices is the vaginal bacteria contamination and inflammation, even when a 6-d short protocol is applied[81,82]. In fact, Manes *et al*[83], observed a decrease of fertility rate in ewes after a 13-days intravaginal permanence of polyurethane sponge impregnated or not with 60 mg of medroxyprogesterone acetate when compared with females with natural estrus and probably due to the vaginal contamination provoked by devices. Oliveira *et al*[81], observed an increase of colony-forming units after a 6-day short-term protocol using intravaginal sponges in goats reaching the highest value at the time of sponge withdrawal, but also a rapid re-establishment of the normal vaginal microbiota. These contaminations seems to be time-based and is more one rational argument for short-term protocols use. The use of local antimicrobial applied to device is a common practice for mitigating vaginal flora[84], but probably the short-term protocols can minimize the degree of vaginal contamination/inflammation if the duration of contact between device and vaginal mucosa is a significant risk factor.

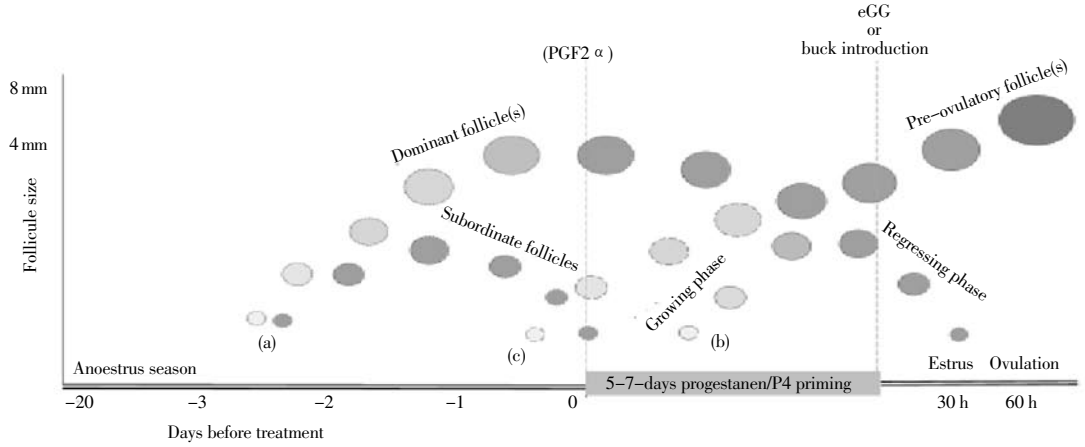

Figure 2. Follicular dynamics in goats using a short progestogen or P4 priming in anoestrous season.
Prostaglandin F2 alfa: PGF2 ; eCG: Equine chorionic gonadotropin; Progesterone: P4. The insertion of a progesterone device can promote the regression of dominant follicle(s) (a) in late plateau phase or regressing phase, and the emergence of a new follicular wave (b). If a young largest follicle(s) is present (growing phase or early plateau phase) at device insertion time (c), it can continues to grow and ovulate. The PGF2 administration should be administered on potential cyclic females, at the onset of treatment, in order to promote the corpora lutea luteolysis and contribute to enhance the follicular wave development. Modified from Rubianes and Menchaca[11].

4. Socio-sexual cues: male effect

The male effect is an interaction male-females, which promote the induction of ovulation in anoestrous females after male introduction in flock (proportion of one buck per 12 goats)[85-87]. Traditionally, females should be isolated from males at least 40 days before male introduction[88]. However, a complete isolation could be not necessary due female interaction regarding not only male sexual activity but also male novelty[87] and novel bucks can induce the male effect even familiar males remained in flocks[45] . Immediately after sexually active male introduction in flock, an increase of plasma LH pulse occurs[89] from anterior pituitary of females, due to hypothalamic GnRH discharges[90]. Bedos *et al* [3] observed an increase of LH pulsatility on anoestrous goats when bucks were introduced in flocks and remained in permanent or intermittent (2 hours per days) contact with females during 5 days, and a decrease of LH pulsatility was observed once the male was removed in the intermittent group. These researchers also observed, in another experiment of the same study, that a similar proportion of goats ovulated when the contact with a sexual active male was 1, 2, 4 or 24 hour of contact per day.

The pre-ovulatory LH peak occurs normally between 1 and 3 days after male introduction and goats ovulate approximately 22 hours after LH peak[91-94]. An intense sexual behavior by male goats is necessary to induce LH preovulatory surge and ovulation[95].

Stimulatory factors are multisensorial and can be classified as fero-hormones, behavior (male-females interactions), and stress factors[96,97]. Probably, all the olfactory, visual, tactile and hearing pathways are involved [86,92,98]. The intensity of male-female interaction is great related with sexual active males presence[99,100]. The previous photoperiod treatment of buck (other than melatonin implant), at least during 30 days, is one natural form to increase the sexual activity of bucks[101]. The previous male sexual experience, recent sexual stimulation with females, novelty of the stimulus are described also as factors that improve the ovulation response[86]. However, social dominance within females[102] and their sexual behavior (ex.: tail wagging)[103], but apparently not their parity[104],

can play an important role.

More than substitute the eCG effect for induction of ovulation in goats without ovulatory activity throughout a single estrus period[92], the male effect also can re-initiate oestrus cycles, anticipating the breeding season[86]. However, male effect is on dependence of both anestrous intensity[86,92] and stimulatory factors[97]. The previous photoperiodic stimulation of females (and males), or the use of melatonin implants, are two effective methods to minimize the anestrus intensity in flocks, and sexually stimulate bucks at latitude 46º N[48] or 37º N[105].

Although a silent ovulation can occurs at first time, inversely to ewes, goats mainly presents estrus behavior. However, normally this first ovulation in goats is followed by a short estrous cycle and a fertile second ovulation event occurs 5 to 7 days after the first one, accompanied by estrus behavior[86,106]. In fact, Delgadillo *et al* [107] observed estrus behavior in 94.7% (18/19) of goats after buck introduction and Lassouet *et al* [108] observed the occurrence of short estrous cycles with a mean duration of (5.6 ± 1.2) days in 100% (20/20) of goats.

In order to increase the synchronization of fertile ovulations, and even to reduce the occurrence of short estrous cycles, a progestogen priming before or at the same time of male introduction can be applied[109]. Véliz *et al* [110] observed that the use of P4 priming can accelerate the response of goats to the buck stimulus. During the first 5 days exhibition of estrus was 70% higher in P4 treated than untreated groups. At 10th day, similar percentages of estrus were observed as well the pregnancy rate after mating. The interval between male introduction and onset of estrus was shorten from (115.0 ± 10.4) h to (64.8 ± 6.1) h using a progestogen priming protocol[111].

At latitude 20º N (Mexico), a 5-day short (0.3g) P4 priming plus male effect was tested with success in 126 dairy goats (French Alpine, Saanen and Toggenburg breeds) during anoestrous season (April and May) even when devices were reused two or three times. Estrous rate (97.5%, 100% and 100%), intervals to estrus (33.8±1.5, 35.2±2.1 and 29.7±1.1 h) and pregnancy rate (62.5%, 79.5% and 69.5%) were not different between groups that used the device for first, second and third time, respectively[102].

López-Sebastian *et al* [113] observed higher pregnancy rate (64.6%), at 40th day, using the IMA.PRO2.1® method when compared with the classical hormonal treatment (pregnancy rate = 46.8%; 45 mg of fluorogestone acetate during 11 days plus 350 IU eCG I.M. and 75 μg cloprostenol I.M. 2 days before sponge removal; AI 46h after), from April to June in Murcia-Spain (L 38º N). The IMA.PRO2.1® method was based in the male effect and a single 25 mg dose of progesterone, I.M., given at the time of buck introduction, for the first ovulation induction. A single 75 μg dose of cloprostenol was administered i.m. 9 days later in order to induce early luteolysis and a new ovulation period. Females were inseminated (dose of 200 × 10^6 spermatozoa in 0.25 mL straws cooled to 5 ºC) 50 h after PGF2 administration.

According to López-Sebastián *et al* [71], other new protocol was tested (Flock-Reprod trademarked progestagen-free protocols) with similar results to the obtained with a classical hormonal treatment. This protocol was based on the application of PGF2 17 days after the buck introduction in flock, i.e. after the short cycle occurrence. However, to our knowledge, results were not yet published.

In conclusion, the use of P4 or progestagens remain crucial for synchronization of fertile ovulation if we want to maximize the fertility during the anoestrous season. However, several recent studies suggest that is possible to reduce the exogenous P4/progestagens exposition on females using short progesterone priming protocols, previous photoperiod treatments in high and middle latitudes, combinations with male effect or even the lower P4 concentration in some devices, including their reutilization. So, endeavors are necessary in order to apply and deepen widely this knowledge.

Conflict of interest statement

Author declare that we have no conflict of interest.

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