RESEARCH ARTICLE

Short term administration of cyproterone acetate for contraception: Effects on testosterone secretion and semen characteristics in rams (*Ovis aries*) and bucks (*Capra hircus*)

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Abstract

Aim of study: To examine the influence of administering cyproterone acetate (CPA), at the beginning of the mating season, on the testosterone concentration and morphometric and functional characteristics of ram and buck semen.

Area of study: Madrid, Spain

Material and methods: Five rams and five bucks were intramuscularly administered 200 mg of CPA in 2 mL of olive oil twice per week - from July 1st to 31st in the rams, and from August 1st to 31st in the bucks. Five control animals of each species were administered 2 mL of olive oil. Blood samples and ejaculates analysed from the start of treatment until eight weeks after the last day of treatment.

Main Results: GLM-ANOVA showed the interaction species × CPA treatment to have effect ($p<0.05$) on sperm motility, progressive motility and acrosome integrity; and greater effect ($p<0.01$) on curvilinear velocity (VCL), straight-line velocity (VSL), viability, and morphological abnormalities. In both the rams and bucks, plasma testosterone levels fell from the first week from the start of CPA administration until three weeks after the end of treatment. In rams, the total sperm count, sperm motility, progressive motility, viability, morphological abnormalities, VCL and VSL were all negatively affected by the treatment ($p<0.001$); acrosome integrity was also affected ($p<0.05$). In bucks, sperm motility, progressive motility, VCL, VSL and morphological abnormalities were negatively affected ($p<0.05$).

Research highlights: Treatment with CPA affected testosterone secretion, semen characteristics and sperm morphometry in both the rams and bucks, and thus it might be used as short term contraceptive protocol in small ruminants.

Additional key words: livestock; male reproduction; ruminants; sperm; testicular activity.

Abbreviations used: CPA (cyproterone acetate); PI (propidium iodide); PNA-FITC (fluorescein isothiocyanate-conjugated peanut agglutinin); TCG (Tris + citric acid + glucose); TTG (Tes + Tris + glucose); VCL (curvilinear velocity); VSL (straight-line velocity)

Introduction

Cyproterone acetate (CPA) [6-chloro-17α-hydroxy-1,2α-methylene-Δ 4,6-diene-3,20-dione-acetate] is the only pharmaco-hormonal compound with anti-androgenic, anti-gonadotropic and progestational properties (Neumann & Berswordt-Wallrabe, 1966; Steinbeck *et al.*, 1971). In males it behaves as an anti-androgenic steroid, acting (i) as a competitive antagonist of androgen receptors, thereby inhibiting testosterone and 5-α-dihydrotestosterone synthesis (Semet *et al.*, 2017), (ii) as an anti-gonadotropic agent that reduces the secretion of luteinizing hormone (LH) and follicle stimulating hormone (FSH), thus suppressing the secretion of androgens (Balbontin &

Ferrario, 1993; Meriggiola *et al.*, 2003) and (iii) its pro-gestational action confers additive and synergistic effects on the ability to suppress gonadotropin levels (Balbontin & Ferrario, 1993; Meriggiola *et al.*, 2003).

The first reports on the functional effects of CPA indicated it to very significantly reduce the fertility of male rats (Whalen & Luttge, 1969); subsequent studies confirmed its impact on reproductive function via the inhibition of spermatogenesis, the perturbation of sperm cell maturation in the epididymis, and by its impairment of the functioning of the accessory sex glands, resulting in a loss of sperm motility and reduced viability (Prasad *et al.*, 1970).

The antiandrogenic properties of CPA have been widely used in urological clinic for treatment of advanced human prostate cancer (Guo *et al.*, 2017) or suppression of sexual disorders (Neumann, 1994). In addition, reversible inhibition of testosterone secretion by the action of CPA (Meriggiola *et al.*, 2003) has allowed its use as a hormonal contraceptive in men; CPA induces a marked reduction in sperm concentration and motility, plus a moderate reduction in the volume of seminal fluid and testicular germ cell numbers (Morse *et al.*, 1973; Koch *et al.*, 1976). Despite that a hormonal regimen consisting of testosterone plus CPA holds promise as an effective, safe and reversible male contraceptive (Meriggiola *et al.*, 1998), questions remain regarding the time required to reach their full contraceptive effect, and the time that elapses between cessation of hormonal contraceptive treatments and full spermatogenesis recovery in men (Khourdaji *et al.*, 2018).

As an androgen inhibitor, CPA has been used in animals for assessing the role of testosterone on antler growth and shedding in fallow deer (Bartoš *et al.*, 2000), red deer (Jaczewski *et al.*, 2004), and white-tailed deer (Bubenik *et al.*, 1987). It has been used in a similar fashion to investigate the role of testosterone on seasonal changes in horn growth in wild Iberian ibexes (Santiago-Moreno *et al.*, 2012), and endocrine relationships between rank related behavior and growth of antlers in deer (Bartoš, 2012). Notwithstanding its strong anti-androgen properties, the literature contains no studies on the use of CPA as an animal contraceptive in non-primates species (Barfield *et al.*, 2006). Effective contraceptive treatments are needed for temporary contraception in wildlife species, and even domestic species, maintained in zoos, wildlife sanctuaries and smaller conservancies where populations need to be controlled, indiscriminate reproduction in mixed herds needs to be avoided, and unwanted aggression needs to be attenuated (Barfield *et al.*, 2006).

Small ruminants native from temperate latitudes show a strong seasonality in their reproductive activity with highest testosterone concentration occurring for a few months throughout the year (Santiago-Moreno *et al.*, 2005; Gómez-Brunet *et al.*, 2012). A potential use of CPA

as contraceptive agent in these species may be expected since early studies showing inhibition of sexual behavior (Fabre-Nys & Signoret, 1980) and a harmful effect on epididymal sperm motility and morphology (Panda & Jindal, 1982) after CPA administration.

Therefore, the aims of the present study were to examine the effects of CPA on semen characteristics in both rams and bucks, and to determine the time that elapses between cessation of contraceptive treatment and the recovery of physiological testosterone secretion. For this purpose we used a short-term protocol of CPA administration during four weeks (Santiago-Moreno *et al.*, 2012). Taking into account the short annual period with high levels of testosterone, and that functional recovery period of testes after CPA interruption appears to be directly proportional to the duration of treatment in rodents (Rastogi *et al.*, 1980), the CPA was administered at the onset of the reproductive season. These results can yield particularly interesting results for reversible contraception in both wild and domestic ruminants.

Material and methods

All the experimental procedures reported here were approved by the INIA Ethics Committee and carried out in accordance with Spanish Animal Protection Policy (RD 53/2013), which complies with EU Directive 2010/63/EU regarding the protection of animals used in scientific experiments.

Cyproterone acetate for administration

CPA (Androcur, Schering A.G., Berlin, Germany) for administration was prepared by dissolving 3.2 g of this compound in 7 mL of benzyl benzoate and then adding 25 mL of olive oil, obtaining a final CPA concentration of 100 mg/mL. This mixture was prepared with agitation at 120°C for 10 min (Santiago-Moreno *et al.*, 2012).

Experimental animals

The study animals were 10 Spanish Merino rams and 10 Murciano-Granadina bucks (all 3 years old), housed in adjacent 250 m2 enclosures under natural daylength and temperature conditions at the Department of Animal Reproduction of the INIA in Madrid (latitude, 40° 25 'N). All animals were fed a balanced diet Visan K59 (Visan Ind. Zoot., Madrid, Spain), which is based on barley grain, barley straw and dry alfalfa. They had free access to water and vitamin/mineral blocks and received routine veterinary treatment for the control of internal parasites.

Experimental procedures

Treatments coincided with maximum testosterone secretion defined for each breed in our latitude, according with previous studies in our lab (Santiago-Moreno *et al.*, 2005; Gómez-Brunet at al., 2012). Five rams and five bucks were intramuscularly administered 200 mg CPA in 2 mL of olive oil (100 mg/mL) twice per week (Monday and Friday), from July 1st-31st (approx. weeks 1-4) for the rams, and from August 1st-31st (approx. weeks 1-4) for the bucks, dates corresponding to the period of increased testosterone secretion during these species annual reproductive cycle (Santiago-Moreno *et al.*, 2005; Gómez-Brunet at al., 2012). This administration protocol helps reduce testosterone in blood plasma to basal (non-reproductive period) concentrations (Santiago-Moreno *et al.*, 2012). Five male control animals of each species were intramuscularly administered 2 mL olive oil without CPA twice per week (Monday and Friday) over the corresponding periods. Sample collection and analyses were performed as described below.

Collection of blood and semen samples, and measurement of scrotal circumference

The entire experimental period lasted 13 weeks. Over the CPA administration period (approx. weeks 1-4), and over the following 9 weeks (*i.e.*, until week 13) blood samples were taken from each animal every Wednesday by jugular venipuncture; samples were collected in 5 mL plastic serum tubes (BD Vacutainer®, Becton Dickinson Co., Plymouth, UK). At the same time, the circumference of the scrotum was recorded at the point of the widest diameter using an orchidometer (Ideal Instruments, Neogen Corporation, Schiller Park, IL, USA). Six ejaculates were also obtained from each animal using an artificial vagina (Cassou IMV Technologies, L'Aigle, France); an oestrus induced ewe was used as a teaser to stimulate sexual behaviour. Samples were collected in 15 mL centrifuge tubes (Sterilin®, Stone, UK). These six samples included one before the start of CPA treatment (week 0), one during treatment (week 2), and four after the last day of treatment, with an interval of 15 days between collections (weeks 5, 8, 10 and 13). All semen samples were transported to the laboratory at 37°C and assessed (all within 30 min of collection).

Blood and seminal plasma testosterone assays

Blood samples were centrifuged at 1500 g for 15 min. Aliquots of the semen samples were left for 3 h at room temperature (18-24°C) and then centrifuged at 2400 g for 10 min. In both cases, the supernatant was removed and stored at -20°C for later analysis. Blood plasma and seminal plasma concentrations were measured by radioimmunoassay in duplicate aliquots (100 µL) as previously described (Santiago-Moreno *et al.*, 2005). All samples were analyzed in a single assay. The sensitivity was 0.05 ng/mL. The intra-assay coefficient of variation was 10.3% (n=10) for the blood plasma analysis, and 11% (n=7) for the seminal plasma analysis.

Semen characteristics assessment

The volume of ejaculates was measured using a micropipette (Gilson, Villiers Le Bel, France). Fractions of the samples destined for assessment were diluted 1:1 (v/v) in TCG solution (313.7 mM Tris, 104.7 mM citric acid, 30.3 mM glucose, 345 mOsm/kg, pH 6.8) for the bucks, and TTG solution (210.6 mM Tes, 95.8 mM Tris, 10.1 mM glucose, 320 mOsm/kg, pH 6.8-7.2) for the rams.

Sperm concentration ($\times 10^6$/mL) was measured in undiluted semen samples using a photometer (SMD1®, Minitube, Tiefenbach, Germany). Sperm motility was determined using a computer-assisted analysis system (CASA) coupled to a model 50i Nikon Eclipse negative phase contrast microscope and using Sperm Class Analyzer (SCA®) v.4.0 software (Microptic S.L., Barcelona, Spain). Samples were diluted 1:200 (v/v) in TTG or TCG medium according to the species, loaded into a 20 µm Leja® chamber (Leja Products B.V., Nieuw-Vennep, The Netherlands) and assessments made at 37°C. We recorded the percentage of motile sperm, and that of sperms showing progressive motility, the curvilinear velocity (VCL; µm/s) and straight-line velocity (VSL; µm/s). All analyses involved the capture of three fields and the examination of 500 sperm tracks at a magnification of 100x (image acquisition rate 25 frames/s).

Acrosome integrity and sperm viability were determined by fluorescence microscopy, analyzing 200 sperm cells, using propidium iodide (PI, Sigma P-4170) and fluorescein isothiocyanate-conjugated peanut agglutinin (PNA-FITC; Sigma L7381) fluorescent probes (Soler *et al.*, 2005). Samples were examined using a Nikon Eclipse E200 epifluorescence microscope (excitation 450-490 nm, emission: 520 nm) (Nikon Instruments Inc, NY, USA).

Morphological abnormalities were assessed by phase contrast microscopy (×400) in samples previously fixed in a solution of 2% glutaraldehyde in BL-1. Two hundred sperm cells were examined, and eight sperm types classified: normal, decapitated, macro/microcephaly, broken neck, defective intermediate piece or double tract, coiled or broken tail, and cytoplasmic drop (Frank, 1950).

Morphometric analyses of sperm heads were performed using samples collected at weeks 0, 8 and 13, employing a model 50i Nikon Eclipse microscope (Nikon Corporation, Tokyo, Japan) with a clear field objective at

60×, and making use of Sperm-Class Analyzer software v.5.3.0.1 (morphometry module) (SCA®, Microptic SL Barcelona, Spain). A total of 100 spermatozoa were analyzed per sample after fixing and staining with Hemacolor®. The width (µm), length (µm), area (µm2) and perimeter (µm) of the sperm heads were determined as previously described (Esteso *et al.*, 2015).

Statistical analysis

Statistical analyses were performed using STATISTICA software for Windows v.12 SP3 (StatSoft, Tulsa, OK, USA). Variables with skewed distributions, as determined by the Shapiro-Wilk test for normality ($p<0.05$), were either arcsine-transformed (for sperm motility, progressive motility, viability, acrosomal integrity and morphological abnormalities) or log-transformed (for blood testosterone levels, total sperm, VCL, VSL and morphometric variables [*i.e.*, non-percentage values]) before analysis. The effect of the interaction species × *cyproterone treatment* on testosterone concentrations and semen characteristics were assessed using general linear model (GLM) repeated measures ANOVA. In the model, the species (buck-ram) and treatment (oil-CPA) were included as categorical predictor variables, and weeks (1-13) as within-subject factors (repeated measures). The influence of the treatment in each species on the testosterone concentration and semen characteristics was assessed by repeated measures ANOVA and the *post-hoc* Tukey test for multiple comparisons. Weekly comparisons between treatment groups and controls were performed using the Student t-test for independent samples. Data were expressed as means±SEM, with the exception of the morphometric variables, which were expressed as means±SDM.

Results

Testosterone concentrations

GLM repeated measures ANOVA showed the interaction species × CPA treatment to have no significant effect on testosterone concentrations.

In the rams, the blood plasma testosterone level was affected quickly and strongly by CPA treatment ($p<0.01$), with differences detected compared to the controls throughout the experimental period ($p<0.05$) (Fig. 1A). Indeed, they were close to the limit of detection during weeks 1 to 7. The first recovery in the testosterone concentration occurred at week 8 (4 weeks after the end of CPA administration). Testosterone levels became fully restored at week 11-12 of the study (7-8 weeks after the end of CPA administration).

In the bucks, blood plasma testosterone was also affected by CPA treatment compared to the controls, with reductions ($p<0.001$) detected throughout the experimental period. Testosterone levels reached their minimum values between weeks 2 and 7. The first recovery occurred at week 8-9 (*i.e.*, 4-5 weeks after the end of CPA administration) (Fig. 1B).

Scrotal circumference

GLM repeated measures ANOVA showed the interaction species × CPA treatment to have effect ($p<0.01$) on scrotal circumference. In the rams, reductions ($p<0.001$) were seen in scrotal circumference at weeks 5 (33.30 ± 1.39 cm *vs* 27.55 ± 0.42 cm), 8 (33.17 ± 2.03 cm *vs* 23.25 ± 0.48 cm), 10 (31.00 ± 1.63 cm *vs* 22.60 ± 0.41 cm) and 13 (36.80 ± 1.86 cm *vs* 27.53 ± 0.14 cm) compared to the controls (with differences detected in the treated animals throughout the experimental period ($p<0.05$)). In the bucks, treatment also reduced the scrotal circumference compared to the controls at weeks 5 (26.28 ± 1.27 vs 23.32 ± 0.40) and 8 (27.03 ± 1.49 *vs* 22.68 ± 0.75) (again with significant differences in the treated animals throughout the experimental period ($p<0.05$)).

Semen characteristics

GLM repeated measures ANOVA showed the interaction species × CPA treatment to have effect ($p<0.05$) on sperm motility, progressive motility and acrosome integrity. A greater effect ($p<0.01$) was found on VCL, VSL, viability, and morphological abnormalities.

In the rams, CPA treatment led to poorer values for the total sperm count, sperm motility, progressive motility, viability, morphological abnormalities and the VCL and VSL compared to controls (all $p<0.001$) from week 2 (normal values were not fully re-established even by week 13) (Fig. 2). Acrosome integrity was also negatively affected ($p<0.05$). Again, differences were detected in the treated animals for all semen characteristics throughout the experimental period ($p<0.05$). The treatment affected to a greater extent at week 8 where four males presented azoospermia.

In the bucks, sperm motility, progressive motility, morphological abnormalities (all $p<0.05$), VCL and VSL ($p<0.01$) were all negatively affected compared to controls (between weeks 2, 5 and 10 for the first two variables, and weeks 2 and 5 for the third) (Fig. 3). Once again, differences throughout the experimental period were detected within the treated group for all semen characteristics ($p<0.05$). Between weeks 2 and 5, four of the five males presented azoospermia.
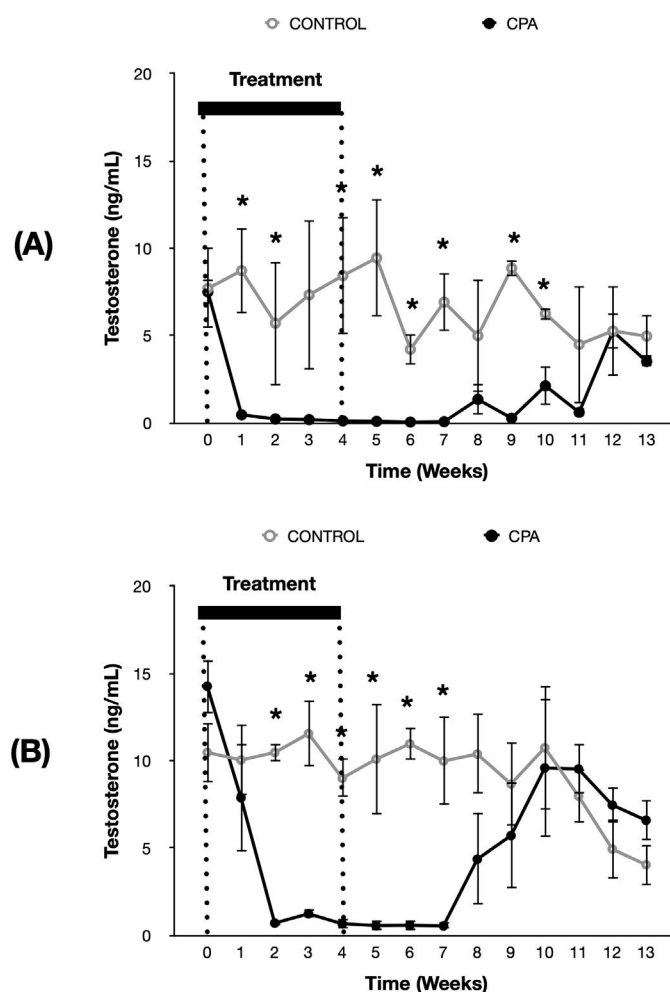
Figure 1. Weekly changes in plasma testosterone concentrations in blood (ng / mL) of Spanish Merino rams (A) and Murciano-granadina bucks (B) (mean ± SEM) in the control (○) and treatment (●) groups during the experimental period. Asterisks indicate a significant decrease (*$p<0.05$) in the treated group. CPA: Cyproterone acetate.

Sperm head morphometry

GLM repeated measures ANOVA showed the interaction species × CPA treatment to have effect ($p<0.05$) on length, perimeter and area of the sperm head. In the rams, the CPA treatment reduced sperm length, area and perimeter from the weeks 8 and 13 of the study period compared to week 0 ($p<0.05$). However, no significant differences were seen between the treated and control groups at any time (Table 1). In the bucks, CPA treatment did not significantly affect any morphometric variable at any time (Table 1).

Discussion

The present results show that, in rams and bucks, treatment with 400 mg/week i.m. CPA for 4 weeks reduces plasma testosterone levels from the first week after the first dose, to 3 weeks after the end treatment. It also reduces scrotal circumference, semen volume and semen characteristics, and affects sperm morphometry. This reduction in plasma testosterone is explained by the anti-androgenic and anti-gonadotropic actions of CPA (Wiechert *et al.*, 1966; Neumann & Töpert, 1986). The rapid reduction in blood plasma testosterone levels in both species is similar to that observed in white-tailed deer (Bubenik *et al.*, 1987), fallow deer (Kolle *et al.*, 1993) and the Iberian ibex (Santiago-Moreno *et al.*, 2012). After the end of CPA treatment, blood plasma testosterone levels in the rams remained at basal levels for 3 weeks; they returned to approximately pre-treatment levels about 8 weeks after the end of treatment. In the bucks, plasma testosterone concentrations were restored by 5 weeks after the end of treatment. This re-normalization time reflects that required for the complete recovery of androgen synthesis by the Leydig cells. For both species, this period is much shorter than the 6-12 months required in humans after
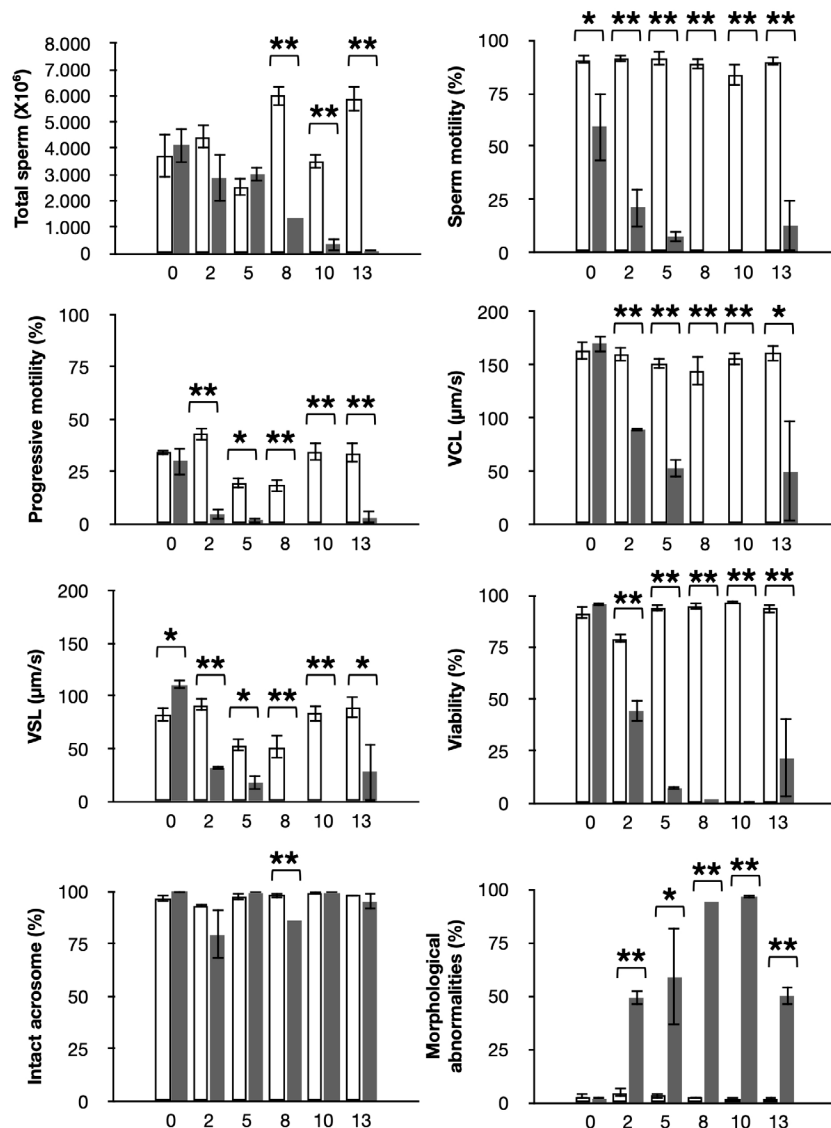
Figure 2. Changes in semen characteristics for the Spanish Merino rams (mean ± SEM) in the control (□) and treatment (■) groups during the experimental period. Asterisks indicate significant differences (*p <0.05, ** *p* <0.01) between groups within weeks. VCL: curvilinear velocity; VSL: straight-line velocity.

treatment with GnRH agonists (Nejat *et al.*, 2000; Shahidi *et al.*, 2001; Kaku *et al.*, 2006). The protocol used in the present study is thus effective in achieving testosterone deprivation for a short time.

The present CPA treatment also induced a significant reduction in scrotal circumference in both species, as reported in other studies (Steinbeck *et al.*, 1971; Balbontin & Ferrario, 1993). The prolonged suppression of testosterone levels beyond the end of treatment may be the direct consequence of changes in testicular histology. Certainly, a positive correlation has been reported between testosterone secretion and the interstitial volume between the Leydig cells, and the testicular lymphatic space in rodents and swine (Mendis-Handagama *et al.*, 1988). Testosterone-related changes in the testicular germinal epithelium, and subsequently in sperm morphometric characteris-

tics, can happen in a relatively short period of time. For example, under physiological conditions, changes in the germinal epithelium in rams occur rapidly at the end of the reproductive season, coinciding with falling testosterone concentrations (Martínez-Fresneda *et al.*, 2019a). In rats, treatment with CPA has a mild inhibitory influence on spermatogenesis; there is no change in sperm tubule diameter, but the size of the accessory glands is strongly reduced (Steinbeck *et al.*, 1971).

The present data for the bucks also suggest CPA treatment to have a mild inhibitory effect on spermatogenesis since sperm concentrations did not fall to values close to zero at any time. In contrast, in the rams, the influence of CPA treatment on spermatogenic activity was much greater: in weeks 11-13 of the experimental period, strong reductions in sperms counts were evident.
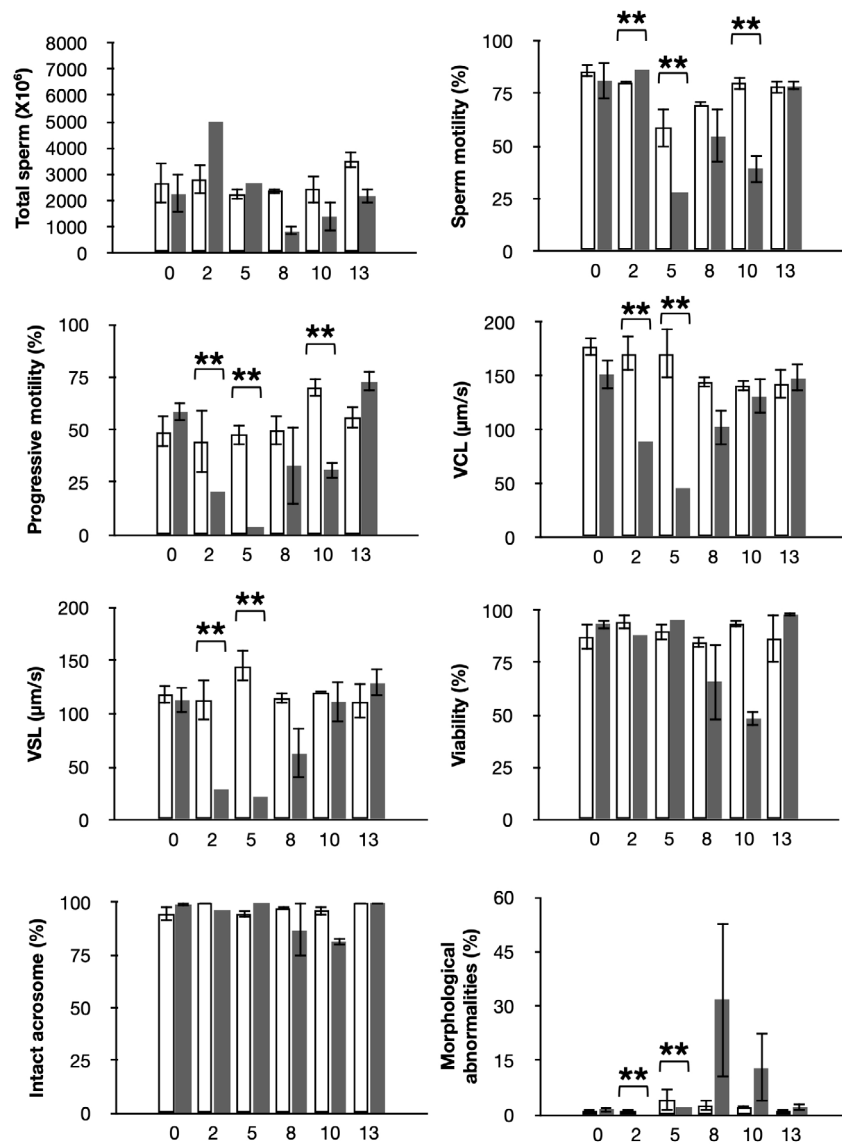
Figure 3. Changes in semen characteristics for the Murciano-granadina bucks (mean ± SEM) in the control (□) and treatment (■) groups during the experimental period. Asterisks indicate significant differences (*p <0.05, ** p <0.01) between groups within weeks. VCL: curvilinear velocity; VSL: straight-line velocity.

Although semen characteristics were affected in both bucks and rams, the extent of damage incurred, and the duration of the temporary contraception effect achieved, differed between the two species. The length of the spermatogenic cycle is very similar in both bucks and rams (about 48 days, França *et al.*, 1999), and thus other causes should explain interspecies differences in the response to CPA treatment, for example differences in their androgen sensitivity. Indeed it is well known that some species respond more strongly to anti-androgen treatment than others (Neumann *et al.*, 1976). This higher sensitivity to CPA treatment in ovine than caprine species occurred despite the similar effect of treatment on testosterone secretion. Inter-species variation in the response to testosterone administration has been previously reported in a recent experiment, showing that frozen-thawed semen characte-

ristics of ram were more sensitive to *in vitro* supplementation with testosterone than buck sperm (Martínez-Fresneda *et al.*, 2019b). However, while sperm quality was affected in both species, spermatogenic activity was not entirely reduced in both rams and goats. While CPA induces testosterone levels similar to those seen outside the reproductive season, it is well known that in these species semen quality falls during this time, but spermatogenesis is not prevented (Arrebola *et al.*, 2010; Martí *et al.*, 2012). Similarly, spermatogenic activity is not entirely arrested in more seasonal bovid species such as mouflons and ibexes (Coloma *et al.*, 2011), unlike cervid species in which spermatogenesis is totally arrested out of reproductive season (Blottner *et al.*, 1996). Treated males of either species were capable to mount and ejaculate before week 7, despite the arrest of testosterone secretion. Maybe,

Table 1. Mean length, width, area and perimeter of ram and bucks sperm heads (mean ± SD).

	Week 0		Week 8		Week 13	
	Control	**CPA**	**Control**	**CPA**	**Control**	**CPA**
Ram sperm						
Length (µm)	8.95 ± 0.26	9.14 ± 0.02[A]	9.07 ± 0.35	8.32 ± 1.17[B]	9.29 ± 0.13	8.61 ± 0.44[B]
Width (µm)	4.76 ± 0.16	4.92 ± 0.08	4.78 ± 0.12	4.52 ± 0.47	4.93 ± 0.03	4.74 ± 0.20
Area (µm²)	35.12 ± 1.74	37.04 ± 0.66[A]	35.77 ± 1.92	31.30 ± 7.50[B]	37.71 ± 0.36	33.76 ± 2.97[B]
Perimeter (µm)	23.62 ± 0.61	24.22 ± 0.20[A]	23.87 ± 0.85	22.08 ± 2.81[B]	24.45 ± 0.23	22.89 ± 1.06[B]
Buck sperm						
Length (µm)	8.61 ± 0.27	8.57 ± 0.21	8.94 ± 0.24	8.78 ± 0.25	8.90 ± 0.09	8.58 ± 0.18
Width (µm)	3.99 ± 0.23	3.94 ± 0.08	4.17 ± 0.14	4.19 ± 0.11	3.96 ± 0.23	4.06 ± 0.06
Area (µm²)	28.59 ± 2.36	27.96 ± 0.13	30.80 ± 1.04	30.43 ± 1.57	29.18 ± 1.36	28.87 ± 0.95
Perimeter (µm)	21.79 ± 0.85	21.65 ± 0.32	22.79 ± 0.45	22.48 ± 0.57	22.31 ± 0.13	21.90 ± 0.35

The capital letters (A-B) indicate significant differences ($p < 0.05$) within each group throughout the experimental period. CPA = Cyproterone acetate

different stimuli such as the contact with active males (control group), the oestrus induced ewe used as a teaser, the routine handling during semen collection sessions, and other visual and olfactory signals in the collection room, stimulated sexual behaviour for mounting (Carrillo *et al.*, 2011; Ungerfeld *et al.*, 2019).

There is a close relationship between the levels of testosterone in blood plasma and the dimensions of the head of human sperm (Garrett *et al.*, 2005). Some authors have reported that during the non-breeding season (characterized by being an endocrine scenario of low plasma testosterone concentrations) ram ejaculates show a reduction in the size of the sperm head (Martí *et al.*, 2012). This is possibly related to changes in the activity of the germinal epithelium and Sertoli cells (Martínez-Fresneda *et al.*, 2019a). The manchette is a transient microtubular platform in elongating spermatids that play a relevant role on the head shaping (Lehti & Sironen, 2016); a certain influence of testosterone levels on the organization of manchette might also explain variations in sperm head dimension.

A close relationship exists between androgen concentrations and semen characteristics (Smith & Walker, 2014). In the present work, CPA treatment reduced sperm motility and viability, and increased the percentage of sperms showing abnormalities. These negative effects are probably explained by the morphological and functional changes experienced by the epididymis as a result of treatment, which include the degeneration of its epithelium and the alteration of its polypeptide profile (Roy & Chatterjee, 1979; Kaur *et al.*, 1992). Additionally, in bucks CPA treatment is reported to have an effect on sperm maturation in the epididymis; the morphological changes seen during CPA treatment may therefore be related to a reduction in the number of mature sperm (Panda & Jindal, 1982). In agreement with previous reports (Roy & Chatterjee, 1979; Kaur *et al.*, 1992), CPA treatment led - in both species - to a significant increase in the percentage of sperms with morphological abnormalities, especially of the decapitated and coiled tail type. Both could be related to alterations in the maturation process in the epididymis during testosterone deprivation. CPA treatment also negatively affected sperm motility (Kaur *et al.*, 1992), perhaps reflecting the cumulative effects of structural and functional damage incurred during spermatogenesis and maturation. The negative effects of CPA on the accessory sex glands, including the involution of the prostate gland and seminal vesicles (Neumann & Kalmus, 1991; Balbontin & Ferrario, 1993) must also affect sperm kinetic activity (Elzanaty *et al.*, 2002). Testosterone in ram seminal plasma fluctuates in a manner similar to that seen in blood plasma (Warikoo *et al.*, 1986; Casao *et al.*, 2010). In binding to sites on the principal piece and midpiece of the tail it seems to regulate kinetic activity. Thus, the fall in testosterone in the seminal plasma caused by CPA treatment could negatively affect sperm motility.

In humans, CPA treatment induces complete azoospermia when combined with testosterone (Meriggiola *et al.*, 1997). The combination of CPA with testosterone should be tested in small ruminants to evaluate its effect on the suppression of spermatogenesis. CPA has been used in animals for manipulation of cycle antler in cervids (*e.g.* fallow deer (Bartoš *et al.*, 2000); red deer (Jaczewski *et al.*, 2004); pudu (Bubenik *et al.*, 2002), but not as a potential contraceptive drug.

Anti-GnRH vaccines have also been used as contraceptive treatments in ruminants. In pygmy goat bucks, such treatment leads to reductions in testosterone concentrations to basal levels, reductions in the size of the accessory sex glands, a reduced sperm concentration, and increases in the proportion of sperms with morphological abnormalities, all for about 6 months. As with the present CPA-treatment, such immunization does not fully prevent spermatogenesis (Martínez-Nevado *et al.*, 2016). However, CPA effects seem rapidly reversible and therefore CPA is more useful as a shorter-term contraceptive treatment. Other contraceptive treatments for ruminants are based on the use of GnRH agonists and subcutaneous implants of deslorelin (which inhibits the reproductive axis for some 17 months), but again, neither cause azoospermia (Giriboni *et al.*, 2019). Contraceptive methods developed so far in small ruminants are not totally reliable to prevent pregnancies. However, taking into account that libido is positively related to testosterone concentrations and considering the large decrease in sperm production and quality, mainly in rams, the fertility would be greatly reduced after CPA treatment.

In livestock production systems, short term contraceptive treatments with CPA can be a useful tool to allow keeping infertile males together with females to facilitate the management in extensive farming, or when seasonal movement of livestock (transhumance) avoiding mating is required. In addition, it may be useful to reduce aggressive behaviour without altering group's social structure (Rosenfield *et al.*, 2019).

In conclusion, treatment with CPA affected testosterone secretion, semen characteristics and sperm morphometry in rams and bucks, and thus it might be used as a short term contraceptive protocol in small ruminants. Although testosterone fell to basal levels, which should induce a lack of libido and promote semen characteristics similar to that observed outside of the reproductive season, spermatogenesis was not fully suppressed in either species, though it was much more strongly inhibited in the rams. Alternative patterns of CPA administration to improve a short term protocol for a reversible temporal contraception in small ruminants should be explored.

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