

Short communication. Synchronization of ovulation and artificial insemination protocol for Spanish ibex (*Capra pyrenaica*) based in progesterone and cloprostenol

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Abstract

This study describes the effectiveness of a method to synchronize ovulation in Spanish ibex for artificial insemination, based on one previously described for use in domestic goats. Estrus and ovulation were synchronised in eight adult ibex females (aged 3-5 yr old) during the breeding season (January) by i.m. injection of 25 mg of progesterone in olive oil plus an 100 µg i.m. of cloprostenol on day 0 followed by a single dose of 100 µg cloprostenol i.m. 9 days later (day 10). Ibexes were inseminated by laparoscopy with 200×10^6 spermatozoa at 52 h after the second cloprostenol injection. Two of the eight treated ibex (25%) conceived and carried their pregnancies to term. The protocol described here minimizes animal handling (in contrast to synchronization methods utilizing intravaginal progestins and equine chorionic gonadotropin injections), which greatly facilitates the application of reproductive technologies in Spanish ibex captive breeding programs.

Additional key words: estrus synchronization, prostaglandins, reproductive technologies, wild ruminants.

Resumen

Comunicación corta. Protocolo de sincronización de la ovulación e inseminación artificial para la cabra montés (*Capra pyrenaica*) basado en la progesterona y cloprostenol

El presente estudio describe la efectividad de un método de sincronización de la ovulación en la cabra montés, para su uso en inseminación artificial, basado en una metodología previamente descrita en la cabra doméstica. El celo y la ovulación fueron sincronizados en ocho cabras monteses adultas (3-5 años de edad) durante la estación reproductiva (enero), mediante la administración i.m. de 25 mg de progesterona vehiculada en aceite de oliva más 100 µg de cloprostenol i.m. en el día 0, seguido de otra inyección de 100 µg de cloprostenol i.m. 9 días después (día 10). Las cabras monteses fueron inseminadas mediante laparoscopia con 200×10^6 espermatozoides, a las 52 h después de la segunda inyección de cloprostenol. Dos de las ocho cabras tratadas (25%) quedaron gestantes y llevaron a término su gestación. El protocolo descrito minimiza la manipulación de los animales (en contraste con métodos de sincronización basados en progestágenos intravaginales y la administración de gonadotropina coriónica equina), lo que facilita en gran medida la aplicación de tecnologías reproductivas en programas de reproducción de cabras monteses en cautividad.

Palabras clave adicionales: prostaglandinas, rumiantes silvestres, sincronización del celo, tecnologías reproductivas.

The Spanish ibex (*Capra pyrenaica*) is an Iberian mountain ungulate that is listed as a threatened species, and thus is an ideal candidate for reproductive technologies that might enhance breeding programmes in this species. Previous studies have reported success in collecting viable epididymal spermatozoa from dead

Spanish ibex males and subsequently artificially inseminating ibex females synchronized by inserting intravaginal progestagens for 11 days in conjunction with injections of equine chorionic gonadotropin (eCG) and cloprostenol two days before progestagen withdrawal (Santiago-Moreno *et al.*, 2006a). Unfortunately, the

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Abbreviations used: eCG (equine chorionic gonadotropin), i.m. (intramuscular), PGF_{2α} (prostaglandin F_{2α}).

pregnancy rate in a captive breeding program is lower than that observed in the wild species (Asher *et al.*, 2000). The occurrence of the low pregnancy rates have been attributed to vaginal inflammation (e.g. vaginitis) by intravaginal progestagens and stress induced fertilization failure (Cameron *et al.*, 1988; Macfarlane *et al.*, 2000). Likewise, the variability in response to hormonal treatments used in synchronization is known to limit fertility (Maurel *et al.*, 2003). Moreover, the presence of anti-eCG antibodies that are typically generated in animals repeatedly treated with eCG (Baril *et al.*, 1996), should be taken into account as a potential problem in captive breeding programmes. Hence, an alternative protocol to intravaginal progestagens and eCG must be found. Since it is most appropriate that female ibex should give birth during their natural time of year (spring), synchronization of ovulation and artificial insemination should occur during their natural breeding season. Thus a method based on injection of luteolytic hormones (PGF_{2α} or its analogues) might be most appropriate. Consequently, a successful method previously described was employed for synchronization of ovulation (IMA.PRO2[®] method; López-Sebastián *et al.*, 2007). This approach minimized the number of times an animal was handled, and avoided the potential of vaginal infections.

The ibex females were inseminated with cryopreserved Spanish ibex epididymal spermatozoa recovered within 8 h after the death of 8 mature ibex males. Epididymal sperm collection and spermatozoa cryopreservation were done according to previous methodologies utilized in our laboratory (Santiago-Moreno *et al.*, 2006a). Briefly, cauda epididymides were isolated from testes and then sperm were collected from the epididymides by performing small cuts in the tubules with a scalpel, and then collecting the fluid emerging from tubules. The sperm rich fluid was then diluted in a media consisting of 3.8% Tris (w v⁻¹), 2.2% citric acid (w v⁻¹), 0.6% glucose (w v⁻¹), 5% glycerol (v v⁻¹) and 6% egg yolk (v v⁻¹). The diluted sperm was then loaded into 0.25 mL French straws and frozen in liquid nitrogen.

The ibex females used in these studies were captured in a National Wildlife Park (37°N, Sierra Nevada, Southeastern Spain) two years before the experiment, and were maintained in captivity in the INIA facilities (Madrid, Spain). The animals were acclimatized to routine restraints and handling. Within the breeding season (January), estrus and ovulation were synchronised in 8 adult females (aged 3-5 yr old) by the i.m. injection of 25 mg of progesterone (4-pregnen-3,20-diona,

Siemsgluss Iberica) in olive oil, plus a 100 µg i.m. injection of cloprostenol (Estrumate[®], Schering-Plough) on day 0 followed by a single dose of 100 µg cloprostenol i.m. 9 days later (day 10). The insemination procedure was as follows. Each ibex female was individually anesthetized using a combination of medetomidine (Domtor[®], Pfizer Inc., 116 µg kg⁻¹ i.m.) and ketamine (Imalgene-1000[®], Rhône Mérieux, 2 mg kg⁻¹, i.m.). A total of 8 insemination straws belonging to 8 ibex males were used to inseminate the ibex females. Each male's fertility had previously been verified by heterologous *in vivo* fertilization (Santiago-Moreno *et al.*, 2006b). Ibexes were inseminated by laparoscopy with 200 × 10⁶ spermatozoa at 52 h after the second injection of cloprostenol. Atipemazole (Antisedan[®], Pfizer Inc., 0.5 mg kg⁻¹ i.m.) was then injected immediately after insemination to antagonize medetomidine-induced sedation. On day 30 after intrauterine insemination, the pregnancy status was assessed by ultrasonography. Pregnant females were allowed to complete gestation, and their gestation length was recorded. Animal procedures were performed according to the Spanish Policy for Animal Protection RD1201/2005 (BOE, 2005), which conforms to the European Union Directive 86/609 (OJ, 1986) about protection of animals used in experimentation.

The synchronization and insemination procedures resulted in two pregnancies (2/8; 25%). The duration of gestation in the two pregnant females were 161 and 162 days. Each pregnancy resulted in the birth of single male offspring.

Synchronization of ovulation using prostaglandins or its analogues requires the presence of active corpora lutea and thus a previous knowledge of the seasonal ovulatory activity of ibex females (December-January; Santiago-Moreno *et al.*, 2003). The ovulatory activity in Spanish ibex females has been characterised, based on a study of luteal cycles in the ibex, which were determined to have estrous cycle lengths of approximately 19 days (range: 15-23 days; Santiago-Moreno *et al.*, 2007). Based on these data, the injection of two doses of cloprostenol within a 10 day interval, during the breeding season, would be an appropriate strategy for synchronization of ovulation in this species. A concern was that social interactions might inhibit cyclic ovulatory activity in captive ibex females (Santiago-Moreno *et al.*, 2007). In the current study, progesterone profiles during synchronization protocol (to minimize stress) were not analyzed; therefore absence of active corpora lutea was to be expected in some treated females. The

synchronization protocol used in this study was based on a successful methodology utilized in domestic sheep (López Sebastián *et al.*, 1988) and goats (IMA.PRO2® method; López-Sebastián *et al.*, 2007), which combined a «ram-introduction» effect plus an injection of progesterone in oil with the synchronization of estrus by the luteolytic effect of a PGF_{2α} analogue (cloprostenol). The rationale behind the injection of progesterone in an oil vehicle was that it would lead to a less variable appearance of behavioral estrus, narrower synchronization of the ovulatory response (López-Sebastián *et al.*, 2007), and better quality of induced corpora lutea (Oldham *et al.*, 1985). This protocol resulted in a higher fertility rate than synchronization methods based on intravaginal progestagens and eCG administration (25.0% vs 16.7%; Santiago-Moreno *et al.*, 2006a). Furthermore, the advantages of this «simplified» method were that vaginal manipulation (and potentially vaginitis) was avoided and the deleterious effects derived from handling stress were minimized. In this sense, it is well known that stress induced by handling during synchronization treatments can lead to delayed or abolished ovulation in some females (Cameron *et al.*, 1988; Macfarlane *et al.*, 2000), or to an early luteal regression (Loskutoff *et al.*, 1990).

In conclusion, the method described in this study should be considered as a viable alternative to synchronization methods based on intravaginal progestagens and eCG injections in captive breeding programs for the Spanish ibex.

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