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Short communication

Fixed-time artificial insemination in red deer (*Cervus elaphus*) in Argentina

J.F. Aller[a,*], O. Fernandez[b], E. Sanchez[a]

[a] *Instituto Nacional de Tecnología Agropecuaria, Estación Experimental Balcarce, Buenos Aires, C.C. 276 (7620) Balcarce, Argentina*
[b] *Estancia San Pedro, Sierra de la Ventana, Buenos Aires, Argentina*

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ABSTRACT

Synchronization of estrous and fixed-time artificial insemination (FTAI) was conducted during the reproductive season of 2008 (March–April) in a local red deer breeding farm in Argentina. Multiparous suckling hinds ($n = 38$) were artificially inseminated following hormonal treatment (intravaginal sponge containing 100 mg of medroxiprogesterone acetate). At the time of sponge removal (day 12) 250 IU of eCG and 500 μg of PGF2α were given to each hind. The FTAI was performed at 48–55 h after device removal with cryopreserved semen imported from New Zealand. Rectal-transcervical AI method (similar to that in cattle) was performed and semen was deposited within the uterine body ($n = 28$) or the cervix ($n = 10$). Pregnancy was diagnosed by means of ultrasonography 44 days after FTAI. The overall pregnancy rate was 36.8% (14/38). Percentage of does that became pregnant with intrauterine seminal deposition was 42.9% (12/28) whereas pregnancy rate in the hinds with intracervical AI was 20% (2/10; $P = 0.27$).

1. Introduction

Red deer (*Cervus elaphus*) is the predominant species used principally for recreative hunting, antler velvet and venison production by the Argentinean deer farming industry. Artificial insemination (AI) has become an important reproductive management tool which allows the use of semen of genetically superior bucks and the introduction of new genetic material into a herd. For estrous synchronization and AI more details concerning female reproduction, such as mating season and length of estrous cycle

* Corresponding author. Tel.: +54 2266 439100; fax: +54 2266 439101.
E-mail address: jaller@balcarce.inta.gov.ar (J.F. Aller).

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are necessary. Hinds are seasonally polyestrous with mating coinciding with decreasing photoperiod in autumn (Thimonier and Sempere, 1989). If hinds do not become pregnant, hinds can continue to have regular estrous cycles until April or the beginning of May in the southern hemisphere. The mean length of the estrous cycle is 18.3 ± 1.7 days (s.d.) in red deer (Guinness et al., 1971) or 19.5 ± 0.3 days (range 10–20 days) in Iberian red deer (*Cervus elaphus hispanicus*) (García et al., 2003). Estrous cycle length of fallow deer (*Dama dama*) was, however, 22.4 ± 1.3 days with a range of 20–27 days (Asher, 1985). Estrous seldom lasted more than 24 h (Guinness et al., 1971).

Laparoscopic intrauterine AI is the most effective method in cervids (Mulley et al., 1988). Other AI techniques such as intravaginal, intracervical and intrauterine insemination have been investigated with varying success (Asher et al., 1993, 2000). Transvaginal approaches would permit more widespread use of AI by producers because these methods are less invasive than laparoscopic AI.

The objectives of the present study were to: (i) examine the efficacy of the rectal-transcervical AI method (similar to that of cattle) and passage rate for AI catheter through the cervical canal and (ii) evaluate pregnancy rate following the intrauterine AI with frozen/thawed semen after hormonal synchronization treatment to induce ovulation.

2. Materials and methods

2.1. Experimental location and animal management

The present research was conducted during the reproductive season of 2008 (March–April) at a local red deer breeding farm located in Buenos Aires province (Humid Pampa), Argentina (37°28′S, 61°56′W). The mean annual rainfall is 800 mm equally distributed in all year. The animals were kept on native pasture and water was provided *ad libitum*.

2.2. Estrous synchronization and artificial insemination

Multiparous suckling red deer ($n = 38$) hinds (4–7 years old) were used. Estrous/ovulation synchronization treatment consisted of insertion of an intravaginal sponge contained within a plastic net (long = 45 cm, INTA Balcarce, Argentina; Fig. 1) and impregnated with 100 mg of medroxiprogesterone acetate (MAP) and 216 mg of oxytetracycline dihydrate (Soler et al., 2007). The sponge device was inserted for 6 days (days 0–6) followed immediately with new replacement device for 6 days (days 6–12). At the time of sponge removal (day 12) 250 IU of eCG (Novormon 5,000, Syntex, Argentina) and 500 μg of Cloprostenol (Ciclase DL, Syntex, Argentina) were given i.m. to each hind. The hinds were fixed-time artificially inseminated (FTAI) one time at 48–55 h after device withdrawal, with cryopreserved semen (0.25 mL straw) imported from New Zealand and obtained from four red deer bucks. Semen straws were removed from liquid nitrogen, thawed in water at 37 °C for 45 s and dried prior to insertion into the insemination device. Only semen from straws with a post-thaw motility of >30% was used for AI.

Rectal-transcervical AI method (similar to cattle) was performed using a cattle embryo transfer device (Transfer Instrument MT®, Minitüb, Germany). The rigid device was inserted into the *os cervix* and manipulated through the cervical canal until either the device passed into the uterine body or no further forward movement was possible. The does were artificially inseminated in standing position without sedation and restrained in the handling cradle. At the time AI, the site of semen placement in the reproductive tract of the hind (intracervical compare with intrauterine) was recorded. The same technician who was experienced in AI and embryo transfer in llama (*Lama glama*) and alpaca (*Lama pacos*) conducted all inseminations.

2.3. Pregnancy diagnosis

Pregnancy diagnosis was conducted using ultrasonography 44 days after FTAI. A real-time mode ultrasonic machine (50S Tringa®, Pie Medical, The Netherlands) fitted with a 5-MHz rectal linear-array transducer was used. Ultrasonic examinations were performed with the hind restrained in standing position. The cable of the transducer (30 cm) was surrounded by a rigid grylon tube for external

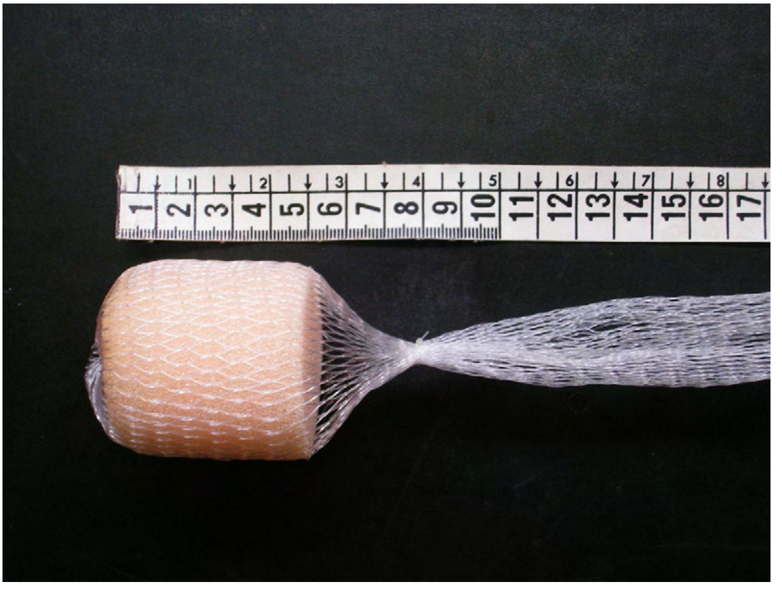

Fig. 1. Intravaginal sponge (100 mg of MAP) contained within a plastic net (long = 45 cm).

manipulations of the transducer in the rectum and held with its longitudinal axis parallel to the hind. Excursions from the midline to both the left and the right side were undertaken to cover the entire uterus. The non-echogenic fluid-filled bladder could be identified immediately as a landmark in almost hinds. Pregnancy was diagnosed by observation of non-echogenic fluid in the uterine lumen or by the identification of one fetus (Fig. 2).

2.4. Statistical analysis

Pregnancy rates of intrauterine and intracervical AI were compared using a two-sided Fisher's Exact Test from Frequency Procedure (SAS, 1989) due to the small number of inseminations.

3. Results

Percentage of hinds inseminated in the cervix or uterus was 26.3% (10/38) and 73.7% (28/38), respectively. The overall pregnancy rate was 36.8% (14/38). Percentage of does pregnant as a result of intrauterine seminal deposition was 42.9% (12/28) whereas the pregnancy rate in the hinds with intracervical AI was 20% (2/10; $P = 0.268$). The pregnancy rates obtained with each buck were 33.3% (4/12), 40% (2/5), 30% (3/10) and 45.5% (5/11).

4. Discussion

Findings in the present research established that red deer hinds can be successfully inseminated by means of rectal-transcervical method (similar to cattle) with an acceptable passage rate through the cervical canal (74%). In fallow does, Willard et al. (2002) used the speculum-guided AI gun and Gourley Scope method (fiber optic technology) and both techniques resulted in an acceptable proportion of intrauterine inseminations (73.1% and 96.3% respectively). Hinds were FTAI at 47–75 h with frozen–thawed semen and the pregnancy rates relative to intracervical and intrauterine semen deposition using speculum-guided AI gun were 42.9% (3/7) and 42.1% (8/19), respectively. The Gourley Scope AI method, however, only resulted in 23.1% (6/26) of does inseminated in the uterus becoming

Fig. 2. Ultrasound image of the uterine horn of a pregnant red deer hinds. Gestational age is 44 days.

pregnant. In the present study, when hinds were artificially inseminated via the intrauterine route pregnancy rate was 42.9%.

Transvaginal/cervical AI following estrous synchronization was performed in red deer hinds using a sheep/goat AI gun and speculum with a light source to aid in visualization of the cervix (Bowers et al., 2004). These authors obtained a pregnancy rate of 41.6% (10/24) with frozen/thawed semen from a single red deer stag. Jacobson et al. (1989), however, achieved a pregnancy rate of 66% with frozen/thawed semen in white-tailed deer (*Odocoileus virginianus*) when inseminations were conducted after estrous detection (mating with a vasectomized buck). A vaginal cannula and small penlight to assist in viewing the cervix during cannula and gun positioning was used in this research. Semen was deposited in the anterior vagina near the cervical opening as the AI device was withdrawn.

An ovarian follicular development synchronization treatment with the CIDR (1.9 g P_4) during 14 days and 200 IU of eCG (day 14) was evaluated in wapiti hinds (*Cervus canadiensis*) (McCorkell et al., 2007). The interval to FTAI was 65 h after device withdrawal. Researchers observed two waves of ovarian follicle development between the time of CIDR insertion and CIDR removal. Ovulations were successfully synchronized in 5/7 (71%) hinds and 4/7 (57%) became pregnant after FTAI with cryopreserved semen. However, the pregnancy rate obtained by Willard et al. (1996) was 42.1% (8/19) in sika hinds (*Cervus nippon*) following insemination with frozen epididymal spermatozoa 60 h after CIDR (0.3 g P_4) withdrawal.

Conception rates to laparoscopic intrauterine inseminations at 54–56 h (red deer) or 65–70 h (fallow deer) after CIDR device removal have been more consistent and generally greater (60–70%) for both species that when inseminations are performed per vagina (intravaginal or intracervical). Conception rates to per vagina inseminations at 48 h after CIDR removal have been poor (less than 40%; Asher et al., 1993). However, a total of 2790 red deer hinds (data collected during commercial operations) were transcervical artificially inseminated (50–62 h after the end of synchronization treatment) and the pregnancy rate was 64% (McMillan and Asher, 2007). Pregnancy rates progressively increased from 51% for "difficult" inseminations to 57% for "moderately easy" inseminations and 65% for "very easy" inseminations. In addition, it was determined that there was no consistent relationship between semen

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quality (percentage of live sperm) after thawing and pregnancy outcomes, possibly due to the large doses of semen used in the previous study (30–40 million sperm per straw).

Umapathy et al. (2007) inseminated 10 spotted deer (*Axis axis*) hinds with fresh semen. For insemination a speculum with a fiber optic light source was inserted to visualize the *os cervix* and a cattle AI device was inserted into the *os cervix*. The interval from norgestomet implant (Crestar, Intervet, The Netherlands) removal to AI was 48–57 h and 3/10 conceived to this insemination.

In summary, development of transvaginal approaches may be acceptable for use in commercial deer farming. With the present research, the rectal-transcervical AI method (similar to that in cattle) resulted in an acceptable proportion of intrauterine inseminations and acceptable pregnancy rate and might be considered appropriate for use in genetic improvement programs in the future in Argentina.

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