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Reproductive evaluation of red deer *(Cervus elaphus)* stags under tropical conditions¤

Evaluación reproductiva del ciervo rojo** (Cervus elaphus) **macho bajo condiciones tropicales

Avaliação reprodutiva do cervo rojo** (Cervus elaphus) **macho sob condições tropicais

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Summary

Background: evaluation of reproductive traits of red deer is important to understand its performance. **Objective:** to evaluate seminal traits of red deer (*Cervus elaphus*) at three different stages of the breeding season (beginning, middle, and end) and to relate semen quality traits with pregnancy rate of hinds. **Methods:** scrotal circumference, semen volume, mass motility, individual motility, sperm concentration, morphology, and intact acrosomes were evaluated in seven stags. After evaluation, each of five stags was bred to 23 to 30 hinds. Pregnancy diagnosis was carried out using ultrasonography 45 days after the breeding season. Data were analyzed using the Student's *t* and chi-square tests, and simple correlation procedures. **Results:** scrotal circumference was reduced 5.4 cm ($p<0.05$) from the beginning to the end of the reproductive season, although semen volume was similar at the three different stages of the season. Sperm concentration (194 *vs.* $622.7/10^6$), mass motility (1.6 *vs.* 2.8), individual motility (28.6 *vs.* 63.3%), and intact acrosome (52.7 *vs.* 75.5%) were greater ($p<0.05$) at the middle of the breeding season in comparison with values found at the beginning. Percentage of abnormal spermatozoa was similar at the beginning and middle of the breeding season ($p>0.05$). No spermatozoa were found in stags at the end of the breeding season. Pregnancy rates were similar among bucks ($p>0.05$), ranging from 80% to 91.3%, and there was no relationship between pregnancy rate and semen traits. **Conclusions:** there was a clear seasonality of semen traits of red deer and no relationship between semen traits and pregnancy rate.

Key words: *breeding season, electro-ejaculation, pregnancy rate, semen traits.*

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Resumen

Antecedentes: la evaluación reproductiva de los ciervos es de suma importancia para entender su comportamiento reproductivo bajo el sistema en el cual se producen. **Objetivo:** evaluar algunos rasgos seminales del venado rojo (*Cervus elaphus*) en diferentes momentos de la estación reproductiva (inicio, medio y final) y relacionar la calidad seminal con la tasa de preñez de las hembras. **Métodos:** se utilizaron siete machos para medir la circunferencia escrotal, volumen seminal, motilidad masal, motilidad individual, concentración de espermatozoides, morfología y acrosomas intactos. Después de la evaluación, cinco machos fueron apareados con 23 a 30 hembras cada uno. El diagnóstico de preñez se realizó por ultrasonografía, 45 días después de la estación de apareamiento. Los datos se analizaron mediante las pruebas de *t* de Student y chi cuadrado, y procedimientos de correlación simple. **Resultados:** la circunferencia escrotal se redujo 5,4 cm ($p<0,05$) del inicio al final de la estación reproductiva, aunque el volumen de semen fue similar en los tres momentos de la estación reproductiva. La concentración de espermatozoides (194 *vs* 622,7/10^6), motilidad masal (1,6 *vs* 2,8), motilidad individual (28,6 *vs* 63,3%) y acrosomas intactos (52,7 *vs* 75,5%) fueron mejores ($p<0,05$) en la mitad de la estación reproductiva que en comparación con los valores del inicio de la misma. El porcentaje de espermatozoides anormales fue similar al inicio y en la mitad de la estación reproductiva ($p>0,05$). No se encontraron espermatozoides al final de la estación reproductiva. Las tasas de preñez fueron similares entre machos ($p>0,05$) variando de 80 a 91,3%, y no se encontró relación entre la tasa de preñez y los rasgos seminales. **Conclusiones:** hubo una clara estacionalidad de los rasgos seminales del venado rojo bajo las condiciones tropicales y una falta de relación entre los rasgos seminales y la tasa de preñez.

Palabras clave: *electro-eyaculación, estación reproductiva, rasgos seminales, tasa de preñez.*

Resumo

Antecedentes: a avaliação reprodutiva do cervo rojo é muito importante para entender seu comportamento reprodutivo no sistema em que eles são produzidos. **Objetivo:** avaliar algumas caraterísticas do sêmen do cervo rojo (*Cervus elaphus*) em diferentes momentos da estação reprodutiva (inicio, meio e final) e relacionar a qualidade seminal com a taxa de prenhez das fêmeas. **Métodos:** foram utilizados sete machos para medir a circunferência escrotal, volume seminal, motilidade masal, motilidade individual, concentração de espermatozóides, morfologia e acrossomas intactos. Depois da avaliação, cinco machos foram acasalados com 23-30 fêmeas cada um. El diagnóstico de gestação foi realizado por ultrassonografia, 45 dias depois da estação de acasalamento. Os dados foram analisados usando provas de *t* de Student e chi-quadrado, e procedimentos de correlação simples. **Resultados:** a circunferência escrotal se reduz 5,4 cm ($p<0,05$) do inicio ao final da estação reprodutiva, ainda o volume de sêmen foi similar nos três momentos da estação reprodutiva. A concentração de espermatozoides (194 *vs* 622,7/10^6), motilidade masal (1,6 *vs* 2,8), motilidade individual (28,6 *vs* 63,3%) e acrossomas intactos (52,7 *vs* 75,5%) foram melhores ($p<0,05$) na parte meia em comparação com os valores do inicio da estação reprodutiva. A porcentagem de espermatozoides anormais foi similar ao inicio e em meio da estação reprodutiva ($p>0,05$). No se encontraram espermatozoides ao final da estação reprodutiva. As taxas de prenhez foram similares entre machos ($p>0,05$) variando de 80 a 91,3%, e não se encontrou relação entre a taxa de prenhez e os rasgos seminais. **Conclusões:** houve uma clara estacionalidade dos rasgos seminais do cervo rojo sob as condiciones tropicais e uma falta de relação entre os rasgos seminais e a taxa de prenhez.

Palavras chave: *electro-ejaculação, estação de acasalamento, rasgos seminais, taxa de prenhez.*

Introduction

Production of Red deer is a novel activity in Mexico. Therefore, it would be of great interest to generate information about productive and reproductive performance of stags and hinds. Reproductive evaluation of stags is important because an unfertile animal can affect an entire group of hinds, reducing pregnancy and lambing rates (Berger, 2000). Reproductive season for Red deer (*Cervus elaphus*) begins at the end of the summer or at the beginning of autumn in the northern hemisphere, terminating at the end of winter, when day length declines (González and Torres, 1998). Under general commercial conditions, the breeding season consists of the introduction of a stag (without any physical or reproductive evaluation) to a group of hinds for approximately 3 to 5 months (Pantoja-Lugo, 2008). In spite of the importance of stags, their reproductive

evaluation is not routinely performed due to their aggressive and nervous behavior (Martinez *et al*., 2008). However, it is possible to obtain stag semen by electroejaculation, which is normally carried out under sedation. Reproductive evaluation of stags brings the opportunity to know the condition of their semen, and allow for the establishing of strategies and management practices for better reproductive use. There are few reports on reproductive characteristics of Red deer stags in the tropics, particularly in Mexico.

The objective of this study was to evaluate seminal traits of Red deer stags at three different stages (beginning, middle and end) of the breeding season and to relate semen quality with pregnancy rate of hinds.

Materials and methods

Location of the study

The study was conducted from July 2009 to January 2010 at the Centro de Desarrollo Tecnológico of Fideicomisos Instituidos con Relación a la Agricultura (FIRA), located in Yucatan, Mexico (20º 09' N y 89º 14' W) at 36 m above sea level. The study complied with bioethics committee norms of the Faculty of Veterinary and Animal Science of the "Universidad Autónoma de Yucatán". The climate of the region is hot sub-humid, with rain in summer (Aw0), 25.8 ºC average temperature, and an average annual rainfall of 1,300 mm (Enciclopedia de los Municipios de México, 2009).

Design of the study

Semen collection was carried out three times (beginning, middle and end) during the Red deer's breeding season. The first semen collection occurred in July, when stags scrub antlers, indicating the beginning of the Red deer's reproductive season in the northern hemisphere (Soler and Cseh, 2009). At that time, stags were dehorned and introduced to a group of hinds (23 to 30). The second collection was carried out 90 days later (October, considered the middle of the breeding season). Collection was conducted when stags were still with the females; it was impossible to separate them due to management problems. The last collection was conducted 90 days after the second one (January, considered the end of the season). Semen evaluation was conducted one week after stags were separated from the hinds.

Animals and management

Seven stags (average age: 8 ± 1.5 years) without apparent physical testicle pathologies were evaluated at the beginning of the study. As one stag died, only six stags were used in the second and third evaluations.

Body condition score of stags was evaluated at the beginning, middle and end of the breeding season, but body weight was taken only at the beginning and end of the season. Body condition was evaluated using a 1 to 5 scale where 1 corresponds to animal emaciation and 5 to animal obesity (Audigé *et al*., 1998). Stags were weighed in the morning (08:00 to 09:00 h) using a SmartScale 300 electronic scale (Gallagher Group Ltd, Hamilton, Hamilton, New Zealand).

Stags were separated from hinds and placed in individual pens on the day of semen collection. Thereafter, they were conducted to a handling facility ("shute") for sedation and injected with 2 mg/kg body weight 10% xylazine i.m. (Pisa Agropecuaria SA de CV, Atitalaquia, Hidalgo, Mexico). Handling of animals was careful during animal sedation, evaluation, and recovery to avoid stress-related complications.

Testes evaluation

Once stags were sedated, the testes, epididymis and spermatic cords were palpated to determine consistency and/or abnormalities. The scrotal circumference was measured using a metric tape while holding the neck of the scrotum and gently forcing the testes down into the scrotum, then putting the measuring tape snugly around the largest circumference. Prepuce and penis integrity was also evaluated. Management was similar throughout the breeding season.

Semen collection and evaluation

Once anesthetized, stags were ejaculated using an electroejaculator (Standard Precision Electronics; Ejaculator System, Inc. Denver, Colorado, USA) with

an electrode for small ruminants (20 cm length and 1 cm diameter) with 3 longitudinal electrodes. The catheter was lubricated and carefully introduced into the rectum with the electrodes directed towards the pelvis. Once located in place, electric stimuli were delivered for 3 to 4 seconds with rest periods of 1 to 2 seconds until ejaculation occurred. Stimuli started at 25 minivolts and gradually increased to 250 minivolts. Ejaculates were collected in a scaled centrifuge tube with a conical end and maintained at 37 ºC. Ejaculates were evaluated using standard semen evaluation procedures for cattle (Evans and Maxwell, 1990; Gisejewski, 2004). Semen volume (Vol) was measured in a scaled collector tube with a conical end. An aliquot was diluted (1:3) in a 1% formalin-saline solution to measure sperm concentration (Conc) at a 1:400 dilution using a haemocytometer (Neubauer chamber). Mass motility (MM; status: 0-5) and individual motility (IM; 0-100%) were assessed by microscope observation at 100X and 400X, respectively. Sperm morphology (Mor) was evaluated in eosin-negrosin smears counting 100 cells per sample. Intact acrosome (IAc) evaluation was performed after fixing an aliquot in a formalin-saline solution and examining 100 spermatozoa using a phase contrast microscope (1,000X). Normal spermatozoa had apical borders with sharp-dark well-defined semilunar shapes (Silva and Gadella, 2006).

Hind management

One hundred and thirty-six hinds without a calf (aged 3 to 10 years) were randomly assigned to five groups (G1, n=30; G2, n=28; G3, n=30; G4, n=23; G5, n=25). One unrelated stag was penned with each group of hinds at the beginning of September 2009 and separated at the beginning of January 2010. Pregnancy diagnosis was conducted 45 days after the breeding season when stags were removed from pens. Diagnosis was conducted using a Pie Medical 100 Falco Vet ultrasound device (Pie Medical Equipment BV, Mastricht, Netherlands) using a linear endorectal transducer (6/8 Mhz).

Statistical analysis

Differences in semen traits (Vol, MM, IM, Conc, Mor, IAc) among season stages (beginning, middle and end) were tested using matched "t" tests. Differences among stags with respect to pregnancy status of their mated hinds were analyzed with Chi-square test. Relationship between semen traits of stags penned with hinds and pregnancy rate was calculated using Pearson simple correlations. All statistical analyses were carried out using the SAS package (SAS Institute, Cary, North Carolina, USA).

Results

Mean body weight and condition score of stags at the three stages of the breeding season are shown in table 1. Stags lost 36.3 kg body weight and reduced 1.1 points in body condition score from the beginning to the end of the breeding season.

Table 1. Body weight and body condition score of Red deer stags at three stages of the breeding season in Southern Mexico.

Traits	Breeding season stage		
	Beginning (n=7)	Middle (n=6)	End (n=6)
Body weight (kg)	183.1 ± 13.7^{a}	-----	146.8 ± 14.0^{b}
Body condition score	4.0 ± 0^{a}	2.5 ± 0^{b}	2.9 ± 0.4^{b}

^{a,b}Different letters in the same row indicate statistical differences ($p<0.05$).

Mean values of scrotal circumference and semen traits of stags are shown in table 2. Scrotal circumference was reduced 5.4 cm from the beginning to the end of the breeding season. Even though high semen volumes were observed at the end of the breeding season, differences with respect to the other two collection stages were not significant ($p>0.05$). Conc, MM, IM and IAc mean values (collected at the middle of the breeding season) were higher ($p<0.05$) than those obtained at the beginning of the season, but the values for normal spermatozoa were similar ($p>0.05$). No spermatozoa were found at the end of the breeding season; therefore, statistical comparisons of sperm traits were not made for this stage.

The overall average pregnancy rate for the five groups of hinds (each group with a stag) was 86.7%. Hind pregnancy rates were similar among stags ($p>0.05$). The lowest value was for stag number 5 (80%) and the highest for stag number 4 (91.3%).

Table 2. Scrotal circumference and semen traits of Red deer stags at three stages of the breeding season in Southern Mexico.

Traits	Breeding season Stage		
	Beginning (n=7)	Middle (n=6)	End (n=6)
Scrotal circumference (cm)	25.2 ± 1.4[a]	22.3 ± 0.9[b]	19.8 ± 1.7[c]
Ejaculate volume (ml)	1.6 ± 0.7[a]	1.3 ±0.7[a]	2.8 ± 1.2[a]
Sperm concentration (x 10^6/ml)	194 ± 168.9[a]	622.7 ± 397.4[b]	0
Mass motility (0-5)	1.6 ± 0.8[a]	2.8 ± 0.4[b]	0
Individual motility (%)	28.6 ± 19.3[a]	63.3 ± 5.2[b]	0
Normal spermatozoa (%)	76.9 ± 17.6[a]	73.5 ± 18.3[a]	0
Intact acrosomes (%)	52.7 ± 14.7[a]	75.5 ± 9.5[b]	0

[a,b,c]Different letters in the same row indicate statistical differences ($p<0.05$).

No association was observed between semen traits and pregnancy rate.

Discussion

Stags had lost 19% body weight by the conclusion of the breeding season. This trend in body weight loss is similar to that reported by Orr (2008), who found a 30% body weight loss. This loss is related to the reproductive activity of stags because they stop eating due to constant activities of courting and breeding hinds; and also because of constant fights to establish hierarchy among stags. Orr (2008) mentioned that weight loss is later recovered during spring and summer. A decrease in body condition score was also observed as body condition score depends on body weight (Orr, 2008).

Scrotal circumference decreases from the beginning to the end of the breeding season, a normal phenomenon in stationary reproductive animals (López *et al*., 1999; Santiago-Moreno *et al*., 2005a). Scrotal circumference is commonly increased at the beginning of the breeding season due to activation of the hypothalamic-hypophyseal gonadal axis which causes androgen-dependent changes such as increase in testicle size (Clarke *et al.,* 2001; Malo *et al.,* 2005; Cassiana *et al.,* 2009), and changes in seminiferous tubules and ejaculate quality (Hochereau-de-Reviers *et al*., 1985; Langford *et al*., 1987; Gastel *et al*., 1995). However, this cannot be demonstrated in this study because scrotal circumference was not measured at the beginning of the study. The short scrotal circumferences at the end of the breeding season could be due to the stag's testicle regression due to a transition period lasting throughout the non-reproductive season, a normal phenomenon in stationary animals (López *et al*., 1999; Santiago-Moreno *et al*., 2005a).

With respect to semen volume, there were no differences among collection stages during the breeding season. The mean ejaculate volume was lower than that observed by Gizejewski (2004), who reported a mean of 2.4 ml. The difference could be partially explained because Gizejewski (2004) collected the semen using an artificial vagina, while an electroejaculator was used in the present study. Furthermore, the first watery transparent fraction was not collected in the present study, and inadequate response of stags to electro-ejaculator stimulus cannot be discarded -causing low volume of semen.

A noticeable increase in sperm concentration was observed from the beginning to the middle of the breeding season; however, no spermatozoa were found in the final stage. Sperm concentration increase was similar to that reported by Gisejewski (2004), who found lower spermatozoa concentration at the beginning of the reproductive season (0.2 to 0.45 x 10^6/mm^3) compared with the middle stage (2.62 to 3.18 x 10^6/mm^3) for Red deer in the Northern hemisphere. The explanation for the high concentration observed at the middle of the season could be that stags were at their peak reproductive levels at this time, resulting from adequate functionality of the hypothalamic-hypophyseal gonadal axis that causes androgen-dependent structural and functional changes and better ejaculate quality (Hochereau-de-Reviers *et al*., 1985; Langford *et al*., 1987; Gastel

et al., 1995). The lack of sperm cells at the end of the season is in accordance with reports by Haigh *et al.* (1984) and Gisejewski (2004). Some authors mention that the lack of sperm cell signals the end of the breeding season due to a decrease in LH and FSH concentration (Gastel *et al.,* 1995; Clarke *et al.,* 2001; Cassiana *et al.,* 2009).

The highest sperm motility (mass and individual) was observed at the middle of the breeding season, which agrees with the results by Gisejewski (2004) who found 60 and 40% values at the middle and beginning of the season, respectively. Malo *et al*. (2005) reported 62% motility at the middle of the breeding season. Greater sperm motility is associated to a larger mitochondrial (Pizzari *et al*., 2002) and endocrine (Santiago-Moreno *et al*., 2005b) activity, with an increase of LH pulses, which favor normal spermatogenesis and development of the accessory glands (Santiago-Moreno *et al*., 2005b).

The lack of difference observed between abnormal spermatozoa (%) at the beginning and middle of the breeding season contrast with results by Gisejewski (2004), who found less than 5% abnormal spermatozoa at the middle and 10% at the beginning of the season. Colas (1984) mentioned that periods of sexual abstinence could decrease ejaculate quality. Abstinence implies a long storage of spermatozoa, which negatively affects the percentage of normal spermatozoa. Malo *et al.* (2005) observed 77% normal spermatozoa in Red deer at the middle of the breeding season, similar to the percentage found in this study.

The high percentage of damaged acrosomes observed at the beginning of the season in comparison with the middle of it could be explained by the reestablishment of spermatogenesis as the breeding season was underway (Colas, 1984; Villarreal, 1999), which is a normal process observed in stationary species, such as the Red deer. Intact acrosomes at the middle of the season was lower than the 90% value reported by Malo *et al.* (2005) and 97% reported by Martinez *et al*. (2008) for the same period. However, Malo *et al*. (2005) used semen obtained from the epididymis. The decrease of intact acrosomes in this study could be due to weather conditions; some studies reported that high environmental temperatures may increase the percentage of abnormal spermatozoa (Marai *et al.,* 2008).

It is worth mentioning that ejaculates without sperm cells were obtained at the end of the season, indicating that stags entered into a sexual activity rest period, as reported by Haigh *et al.* (1984) and Gizejewski (2004), who also observed ejaculates free of sperm at the end of the period of sexual activity of Red deer stags.

Pregnancy rate

No differences between pregnancy rates of the different groups were observed in the present study. Correlation of pregnancy rate and semen traits was not significant. Pregnancy rates (80% to 91%) were higher than the values found by Gomendio *et al.* (2007), who reported 24 to 70% fertility rates using natural breeding. They were also higher than values reported by Malo *et al*. (2005), who reported 20 to 75% pregnancy rates using artificial insemination. The high pregnancy rate found here could be due to the long breeding season (September to January), which allowed more copulation opportunities. Several researchers mentioned that individual differences among Red deer stags could contribute to differences in reproductive performance of hinds (Malo *et al.,* 2005; Santiago-Moreno *et al.,* 2006). Some authors mention a relationship between pregnancy rate and semen quality (Malo *et al*., 2005; Gomendio *et al.,* 2007). However, this study does not support that claim; stags with low semen traits had better pregnancy rates in this study.

In conclusion, there was a clear effect of breeding season stage on semen traits of Red deer under tropical conditions, but a lack of relationship between semen traits and pregnancy rate was also observed. These results suggest that pregnancy in hinds is the result of many factors, one of which is semen quality. However, the ability of stags to seek the hinds with greater receptivity and reproductive characteristics was not taken into consideration in this study, which is a very important aspect for obtaining high pregnancy rates.

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