Short communication. Real-time ultrasonography for early pregnancy diagnosis and incidence of embryonic/foetal mortality in farmed Iberian red deer hinds

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Abstract

The main objective of the present study was to evaluate the accuracy of the trans-rectal ultrasonography technique applied to the Iberian red deer hinds since it has not been previously documented in Spain. Experimentation was also used to test the hypothesis that a lower fertility in young hinds could be due to an increment in embryonic or foetal mortality. For this purpose, 116 hinds divided in three groups depending on its age (young, adults and senescent) were examined by rectal ultrasonography and the different foetal structures measured two months after the males were removed from the groups of hinds. With these data, specificity and predictive value of negative diagnoses of the technique has been calculated. Trans-rectal ultrasonography is an accurate technique to use in Iberian red deer dams with overall levels of pregnant detection near to 92%. Finally, the youngest hinds showed poorer fertility rates (17.24% *vs.* 59.30% and 58.33% of senescent and prime-age respectively) and also higher of embryonic or foetal mortality (2 deaths out of 7 positive pregnant diagnoses compared with no deaths in the senescent group and 4 deaths out of 39 positive pregnant diagnoses in the prime-age group).

Additional key words: echography; fertility; reproduction; Spain.

Resumen

Nota corta. Aplicación de la ultrasonografía transrectal en el diagnóstico precoz de gestación y evaluación de la mortalidad embrionaria/fetal en ciervas ibéricas mantenidas en granja

El objetivo principal del presente estudio ha sido evaluar la precisión de la ultrasonografía transrectal aplicada a las ciervas ibéricas, ya que no ha sido documentado previamente en España. Paralelamente, se ha intentado comprobar la hipótesis de que la menor fertilidad en las hembras más jóvenes podría deberse a una mayor mortalidad embrionario-fetal. Para ello, 116 hembras divididas en tres grupos en función de su edad (jóvenes, adultas y viejas) fueron ecografiadas a los 2 meses de la retirada de los machos y medidas las diferentes estructuras fetales. Con estos datos se ha calculado la especificidad y seguridad en el diagnóstico negativo de la técnica. La ultrasonografía transrectal es una técnica precisa para su uso en ciervas ibéricas, con niveles de detección de gestación cercanos al 92%. Finalmente, las ciervas más jóvenes muestran peores índices de fertilidad (17,24% *vs* 59,30% y 58,33% del grupo de viejas y adultas respectivamente) y mayores mortalidades embrionario-fetales (2 muertes de 7 diagnósticos gestantes positivos en comparación con ninguna muerte en el grupo de hembras de más edad y con 4 muertes de 39 diagnósticos gestantes en las hembras adultas).

Palabras clave adicionales: ecografía; España; fertilidad; reproducción.

Real-time ultrasonography has been used to determine the status of pregnancy in several species of farm animals (Reichle and Haibel, 1991; Aiumlamai *et al.,* 1992). Trans-abdominal ultrasonography is considered a reliable technique in early pregnancy diagnoses in fallow deer (*Dama dama*) does that are more than 50 days pregnant (Mulley *et al.,* 1987). However, as in most of the deer farms crushes are not available and it is not provided adequate access to the does' ventral abdomen, trans-rectal ultrasound scanning is the method of choice in this species. Ultrasound pregnancy testing of red deer using a rectal probe was first investigated by Bingham *et al.* (1988) and Revol and Wilson (1990). They confirmed that the pregnancy status of red deer could be determined with a very high degree of accuracy (>98%) between 30-130 days of pregnancy. Given that in most red deer populations, the probability of being fertile varies essentially within the yearling females (Langvatn *et al.,* 1996; Ansorge *et al.,* 1999) and that the relative risk of yearlings over adult hinds of losing their calves in early pregnancy is 1.87 (Audigé *et al.,* 1999a), a lower fertility in young hinds could be due by an increment in embryonic/foetal mortality. The present study has been made to test this hypothesis. At the same time and because the trans-rectal ultrasound scanning is used routinely in countries where red deer is now an alternative livestock species, but has not been previously documented in Spain, the accuracy of the technique has been evaluated applied to the Iberian red deer hinds.

Subjects were 116 Iberian red deer hinds from the experimental farm of *Escuela Técnica Superior Ingenieros Agrónomos* of Albacete (Spain) divided in three mating groups depending on its age. Group 1, senescent, compounded by 27 does born between years 1993 and 1999, *i.e.* hinds between 10 and 16 years of age. Group 2, adults or "prime-age", formed by 60 hinds born between years 2000 and 2006 (between 3 and 9 years old). Group 3, young hinds, compounded by 29 does born between years 2007 and 2008 (hinds between 1 and 2 years old). The animals were not subjected to hormonal synchronization treatments and were naturally mated. For this purpose, on August 27th 2009, hinds were divided in three groups for breeding so that each group was assigned to five stags which were maintained during a month and removed from the herds on September 29th. Two months after the males were removed from the groups of hinds, these were examined by rectal ultrasonography using a ultrasonic equipment Aloca 500 SSD with a 7.5 MHz probe while they were standing. Each scan was recorded on video tape for measurements of foetal structures. Criteria to assess the accuracy of trans-rectal sonography were based on the results of the following indexes (Kahn *et al.,* 1993): i) specificity [(No. of correct diagnoses "non-pregnant" / No. of hinds non-pregnant) * 100] and ii) predictive value of negative diagnoses [(No. of correct diagnoses "non-pregnant" / No. of hinds diagnosed non-pregnant) * 100]. Using the computer package SPSS 15.0 (SPSS, Chicago, IL, USA) statistical comparisons were examined by cross-tabulations with Chi-squared statistic (Agresti, 1990) for differences among groups in reproductive efficiency. To compare differences between groups in embryonic or foetal deaths as 80% of the expected frequencies not exceeded 5, Fisher's exact test was used.

The indexes of the efficacy of this technique are shown in Table 1 for each group. Taking together (for the three age classes combined) the predictive value of negative diagnosis was 94.50%. Fertility rate was globally 48.30%. By age groups, fertility rates were 59.30%, 58.33% and 17.24% for senescent, adults and young hinds respectively. In this regard, statistically significant differences were observed between the group of young females and the other two groups, both adult females and senescent (χ^2: 13.34, $p < 0.001$ and χ^2: 10.53, $p < 0.001$ in relation to adult and mature groups, respectively), where the OR (odds ratio) between young and adults were 6.72 (95% confidence interval, CI_{95}: 2.26 to 20.02) and between young and senescent 6.98 (CI_{95}: 2.04 to 23.93). By contrast, there were no statistically significant differences between groups of adults and senescent hinds. In relation to embryonic or foetal mortality according to age group, only marginally significant differences ($p = 0.083$) were observed between the group of young females, with 7 hinds tested pregnant which 5 out of them finally calved (2 embryonic/foetal deaths) and the mature

Table 1. Results of accuracy indexes of the trans-rectal ultrasonography in three groups of Iberian red deer hinds

Group[1]	Specificity (%)	PVD[2] (%)
1	100	81.8
2	84.0	95.5
3	91.7	100

[1] Group 1: hinds between 10-16 years old (senescent). Group 2: hinds between 3-9 years old (adults). Group 3: hinds between 1-2 years (young). [2] PVD: Predictive value of negative diagnosis.

females with no embryonic/foetal death (16 calving hinds of 16 positive pregnancy diagnoses). No differences were found between the group of prime-age hinds (39 hinds tested positive pregnant which 35 out of them finally calved, *i.e.,* 4 embryonic/foetal deaths) and the groups of young or senescent hinds.

Since positive diagnosis was made by direct observation and measurement of foetal structures (crown-rump length, head length, head diameter, nose length, chest depth, chest width) then we considered that sensitivity was 100% like those data reported by other authors (White *et al.,* 1989; Bingham *et al.,* 1990). Anyway, the degree of specificity and accuracy of our results can be considered high and this indicates that from two to three months gestation onwards the transrectal technique can be used very reliably. Since it seems that the value of scanning the animals much before the fourth week of gestation is doubtful in terms of reliable pregnancy diagnosis (Fennesy *et al.,* 1986), other authors coincide in about two months of gestation as the best time to carry out the scanning (Smith and Lindzey, 1982). In contrast, during the second trimester of pregnancy (from week 14 onwards), the foetal characters were often difficult to measure, as the foetus had descended too far into the abdominal cavity so that this technique is effective until approximately 120 days gestation considered the end of the useful period to carry out it (Smith and Lindzey, 1982; Bingham *et al.,* 1988).

The results of fertility are lower to those reported in studies with hinds naturally mated. Thus, adult hind pregnancy percentage of 96.8% have been reported in red deer herds (Audigé *et al.,* 1999b) and our own results have always been above 90% but keeping males 2 months in the herds (Carrion *et al.,* 2009). In this sense, in the Iberian subspecies cyclic luteal activity is found in all the population from October to February (García *et al.,* 2002) and the stags of this experiment were removed at the end of September. There was a strong effect of age on fertility, showing these results that the probability of being pregnant is approximately 7 times higher for prime-age or even senescent hinds than for the younger ones. In this case there were no differences between the low fertility of yearlings (only one pregnant out of 11, which subsequently failed to calve) and those of 24 months (just 4 calved out of 15). The effect of age on fertility has been observed by some authors (Albon *et al.,* 1986; Carrión *et al.,* 2009) which show that clearly the does have more difficulties of being pregnant within their

first year and that the yearlings are being pregnant later than the older animals, and even if they do not reach a certain body mass and development, diminishing the likelihood of conceiving that year (Albon *et al.,* 1986). In this sense, puberty attainment and the maintenance of female reproductive ability seems to be dependent in most of the mammal species not on the chronological age but on the body condition associated to that age and more closely on the attainment of particular threshold of fat to lean mass than on a critical body mass (Frisch, 1984; Flueck, 1994). However, the effect of body weight on fertility seems not probable to explain the differences on fertility in the youngest females since as these were maintained under farm conditions their body weight and body condition were enough to be fertile. A more reasonable explanation rests on the fact that the youngest hinds are reproductive active later in the breeding season compared with the adult ones (Albon *et al.,* 1986) and if they were pregnant in a lesser proportion, could be due because the sire bucks were kept within the hinds just for a month, not enough time to allow stags to mate all the yearling hinds coming into oestrus at the end of the rut. So, early remove of sire stags to yearling hinds had a negative influence on conception. In any case, the effect of age on conception rates is not easily explained and it may be too related to social factors. Young hinds are likely to be low in the social rank and consequently more often harassed by dominant hinds and the stress imposed upon these young hinds may have a negative impact on ovulation (Audigé *et al.,* 1999c). Two out of 7 hinds diagnosed as pregnant between the youngest dams failed in the posterior calving which was sufficient to be marginally significant in comparison with the group of senescent hinds. These results suggest a possible effect of age on embryonic/foetal mortality but, given the scarce number of cases, further analysis will be necessary to elucidate this fact.

In any case, energy costs of ovulation and fertilization are minimal compared to those for gestation and lactation (Clutton-Brock *et al.,* 1989) and lactation puts yearling hinds under higher nutritional stress than adults thus lowering their body condition disproportionately (Audigé *et al.,* 1999a). It is possible that embryonic/foetal mortality could be a mechanism whereby the doe could adjust her level of reproductive effort in mid cycle, but prior to substantial investment, to correspond more closely to her capacity to invest in gestation and lactation.

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