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Factors influencing reproductive efficiency and pregnancy in Pura Raza Española mares

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ABSTRACT: The aim of this study was to analyse ovarian activity and pregnancy in 140 Pura Raza Espanola mares over a period of two consecutive years. The data were analysed using SPSS software to evaluate the effects of age, category of mares and season on pre-ovulatory follicle size, ovulation rate, interval from foaling to pregnancy, gestation length, incidence of twin pregnancies and pregnancy losses. The results showed that follicular diameter was significantly ($P < 0.05$) smaller in fillies (3-year-old) and maiden mares than in older and multiparous mares. Within the group of animals treated with gonadotropin hormone hCG, 87.23% ovulated 48 h following administration. Length of gestation varied from 305 to 356 days and all gestation pregnancies of less than 305 days resulted in a non-viable foal. In addition, foaling season affected ($P < 0.05$) the post-partum conception interval and the length of gestation, which were shorter and longer in the winter foaling, respectively. Regarding the other parameters, mares of this stud exhibited relatively very low rates of double ovulations, twin pregnancies and pregnancy losses. We conclude that age and category of mares and foaling season influence fertility and foaling rates by affecting pre-ovulatory follicle size, pregnancy loss and gestation length.

Keywords: mare; ovulation; pregnancy loss; post-partum; foaling; hCG; twin pregnancy

Horses have long been one of the most important domesticated animals, helping to transport people and cargo. Today, horse breeding is a major business sector that provides more than 100 000 direct and indirect jobs in Europe (European Horse Network 2010). Veterinarians and stud owners are constantly seeking ways to improve stud performance. To achieve this objective, it is important to optimize the management of each herd, not only in relation to housing, feed and the well-being of the animals but also by acquiring in-depth knowledge on the reproduction of each individual horse breed.

"Pura Raza Española" or Andalusian horse (PRE) is probably the most important horse breed in Spain and one of the oldest breeds in the word. However, despite the fact that it has spread throughout the world since the 15th century, only a small number of reproduction studies have been performed in this horse breed (Akourki et al. 2013).

Several studies have reported that factors such as double ovulations and multiple pregnancies that are responsible for conceptus loss (Merkt and Jochle 1993), depend on mare age (Davies Morel et al. 2005), breed or type of stud (Nath et al. 2010), the season (Davies Morel and Newcombe 2008), and the use of gonadotropin hormones (Veronesi et al. 2003). On the other hand, the interval from foaling to gestation and gestation duration have been associated with the gender of foal conceived (Davies Morel et al. 2002; Perez et al. 2003; Valera et al. 2006), the age and parity of the mare (Demirci 1988; Valera et al. 2006), and foaling or mating season (Nagy et al. 1998; Davies Morel et al. 2002; Heidler et al. 2004).

Recent studies showed that spontaneous embryo death and early pregnancy loss, which are major causes of economic losses for the farmer, as well pregnancy rates at foal heat, can increase with the

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age of the mare (Sharma et al. 2010; Schulman et al. 2013).

It is hypothesised that ovarian activity and pregnancy losses in PRE are influenced both by diverse mare factors as well as by the season. The study was focused on the effect of (I) age and category of females and (II) foaling season on reproductive outcomes over a period of two consecutive years in PRE mares.

MATERIAL AND METHODS

We studied the reproductive parameters of a total of 140 PRE mares at a stud farm located in El Bayo, Huesca, North-East Spain, in 2007 and 2008. The studied females were classified as maiden, barren or lactating mares. The general management of the animals was as follows: all of the mares were kept indoors in large pens where they received a diet based on oats, lucerne and hay and underwent a disease control program that comprised de-worming and vaccination. Each female was vaccinated annually against tetanus and influenza and pregnant mares were also vaccinated against equine herpesvirus type 1 and type 4 at five, seven and nine months of gestation.

For reproductive management, first, an uterine lavage with a saline solution was performed three to four days post-partum for each mare in order to prepare the uterus for hygienic inseminations at foal heat. After this step, the reproductive tract of the mare was scanned regularly to follow growing follicles using an electronic Honda HS-2000 ultrasound scanner, fitted with a 7.5 MHz linear probe. When follicle size reached a diameter of 40 mm, the female was inseminated and injected with 3000 IU of chorionic gonadotropin hormone (hCG, Veterin Corion, Divasa Farmavic, Barcelona, Spain). Mares with a preovulatory follicle size greater than 50 mm, were not injected. After this first insemination, the mares were scanned daily to evaluate follicle size and were inseminated every two days until ovulation occurred. The ovulated follicle in the ovary can represent single left (L) or right (R) ovulation, double ovulation in the left (L + L) or right (R + R) ovary or in both ovaries (L + R). Pregnancies were confirmed using transrectal ultrasonography on Days 14, 30, 60 and 90 post-insemination. When a mare was detected as not pregnant on Day 14 post insemination, we repeated this protocol of hormonal treatment and insemination until a pregnancy was obtained. For reasons related to the stud, twin pregnancies were always allowed to carry to term. Embryo mortality was detected on Day 30 using ultrasound scanning and foetal mortality was verified by an echography on Days 60 and 90 post-insemination.

For this study, the mares were grouped into three groups according to their last foaling date: January–March (winter), April–June (spring) and July–September (summer). The age of all of the females ranged from three to 23 years, and the mares were divided into three age groups; Group 1: 3-year-old female mares or fillies, Group 2: 4- to 10-year-old and Group 3: more than 10 years old.

The following parameters were evaluated: by regular follicle scanning using the ultrasound scanner, the size or diameter of the preovulatory follicle (mm); birth to pregnancy interval (days); duration of gestation (days); incidence of double ovulations and twin pregnancies (%) and embryonic loss rate (%), which denoted resorbed embryos in the first three months of gestation and aborted foetuses after this date.

For statistical analysis, data of 140 reproductive mares were processed using statistical software (SPSS 15.0 Windows). The main effects analysed were the following: reproductive mare category (maiden, barren or lactating mare), active ovary (L, R, L + L, R + R and R + L), female age (Group one, two and three), the previous foaling season (winter, spring and summer) and foal gender (male or female) on follicular activity and/or on the pregnancy parameters (gestation length, incidence of double pregnancy, pregnancy loss and live birth rates). We used ANOVA and Duncan's test to compare multiple means. Additionally, we applied the non-parametric binomial test to evaluate the effect of hCG injection on the ovulation rate at 48 h and to study the influence of type of heat (foal heat or other) on the pregnancy rate.

RESULTS

Reproductive events

Among the 140 mares included in this study, all were pregnant and 132 mares delivered in the first season. However, of the 132 females studied in the second season, only 79 delivered (Table 1).

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Table 1. Numbers of mares inseminated, pregnant and the number of deliveries in the first season

	First season	Second season
Inseminated mares	140	132
Pregnant mares	140	79
Deliveries	132	79

Ovarian activity

The results showed that the size of the preovulatory follicle is affected not only by the mare category and age but also by ovarian activity regardless of the season evaluated. The ovulated follicle was significantly ($P < 0.05$) smaller in maiden and younger mares than in mares that had delivered and older mares (Tables 2 and 3). There was no significant difference in preovulatory follicle size between ovaries situated on the left or right side in single ovulations: the values were 47.7 vs 48.5 mm for right and left ovaries, respectively. However, in the case of double ovulations there was a tendency for a reduction in the follicle size. In the same way, the preovulatory follicle size was the smallest ($P < 0.05$) when double ovulation occurred in the same ovary: 42.33 mm (L + L ovaries) vs 45.5 mm (R + L ovaries).

The single ovulation rate resulting from these follicles was 53.43% (70/131) for the L ovary and 46.57% (61/131) for the R ovary, whilst with double ovulation, the rate was 66.67% (6/9) for the right (R + R) ovary and 33.33% (3/9) for the left ovary (L + L). This double ovulation rate of 6.42% (9/140) gives a total of 1.42% (2/140) twin pregnancies.

When all double ovulations were analysed, 77.78% (7/9) resulted in single pregnancies and 22.22% (2/9) in twin pregnancies, with live birth rates of 85.71% (6/7) and 50% (1/2), respectively.

Analysis of the effect of the hCG gonadotropin hormone on ovulation revealed that 87.14% of treated mares ovulated 48 h after hCG application; this percentage was significantly higher ($P < 0.05$) than non-responders (12.85%).

Gestation parameters

Having given birth once, the time it took for mares to conceive again varied between nine to 69 days (mean: 28.34 ± 15.76 days). This time did not differ depending on the season of foaling (Table 4). For the pregnant mares, 39.28% were pregnant at first oestrus post-partum and the other 60.71% conceived from the second to third oestrus.

The total length of gestation varied from 305 to 356 days (mean: 327.22 ± 10.94 days) and mares started to bear viable offspring from Day 305 onwards. Length of gestation did not vary depending on the age and category of the mare (Tables 2 and 3). However, this parameter was significantly influenced ($P < 0.05$) by the season in which the female had had its previous delivery; the mares that foaled during the last winter had a longer gestation period compared to those that foaled during summer (Table 4). In addition, this parameter was affected ($P = 0.011$) by the gender of the offspring and was 327.47 ± 7.02 days for female foals and 329.67 ± 9.81 days for male ones.

The rate of pregnancy loss was 5.71% (8/140); 87.5% (7/8) were represented by resorbed embryos in the first three months of gestation and 12.5% (1/8) corresponded to aborted foetuses after this date. Pregnancy loss was also dependent on mare category and age and on foaling season. Therefore, rates of pregnancy loss were 87.5% (7/8), 12.5%

Table 2. Effect of mare category on the size of the preovulatory follicle and length of gestation in the first season. Data are presented as mean ± SD (number of animals)

Mare category	Size of preovulatory follicle (mm)	Length of gestation (days)
Maiden mare	43.00 ± 3.01^{b} (15)	328.50 ± 14.84^{a} (14)
Barren mare	47.69 ± 4.72^{a} (45)	330.80 ± 8.66^{a} (45)
Lactating mare	49.02 ± 4.99^{a} (80)	325.72 ± 11.50^{a} (73)
Total number of mares	140	132

^{a,b}Values in the same column bearing different superscript letters are significantly different at $P < 0.05$

Table 3. Influence of age of mares on follicular diameter and length of gestation in the first season. Data are presented as mean ± SD (number of animals)

Age of the mares (years)	Size of preovulatory follicle (mm)	Length of gestation (days)
Group 1 (3)	43.73 ± 3.17^{a} (15)	329.60 ± 8.90^{a} (14)
Group 2 (4–10)	48.50 ± 4.85^{b} (92)	326.32 ± 9.45^{a} (86)
Group 3 (10)	48.78 ± 5.31^{b} (33)	329.63 ± 15.44^{a} (32)
Total number of mares	140	132

^{a,b}Values in the same column bearing different superscript letters are significantly different at $P < 0.05$

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Table 4. Effect of season on follicular diameter, length of gestation and post-partum interval to conception of mares delivered in the second season. Data are presented as mean ± SD

Season	Size of preovulatory follicle (mm)	Length of gestation (days)	Post-partum interval to conception (days)
Winter	53.83 ± 4.53^{a} (11)	333.83 ± 9.53^{a} (11)	20.50 ± 17.45^{b} (11)
Spring	48.58 ± 4.69^{b} (58)	327.92 ± 9.35^{ab} (57)	32.30 ± 15.72^{a} (57)
Summer	48.30 ± 4.96^{b} (11)	322.20 ± 7.08^{b} (11)	30.60 ± 16.27^{a} (11)

^{a,b}Values in the same column bearing different superscript letters are significantly different at $P < 0.05$

(1/8) and 0% (0/8) for lactating, maiden and barren mares, respectively, and 12.5% (1/8), 75% (6/8) and 12.5% (1/8) for mares aged three, four to 10 and more than 10 years old, respectively. Values for foaling season were 25% (2/8) in winter, 12.5% (1/8) in summer and 62.5% (5/8) in spring.

DISCUSSION

In this study, several factors were shown to affect ovarian activity in PRE mares. We observed that the diameters of double unilateral follicles were smaller than those of two single bilateral follicles or a single unilateral preovulatory follicle. These results are in agreement with those reported by Ginther and Pierson (1989). Higher numbers of ovulations were recorded in left ovaries than in right ones, confirming the findings of previous studies, such as those of Ginther (1983).

In our study, PRE mares exhibited a double ovulation rate of only 6.42% with 1.42% twin pregnancies, much lower than the percentages previously reported by other authors in different horse breeds (Ginther et al. 1982; Merkt and Jochle 1993; Veronesi et al. 2003; Davies Morel and Newcombe 2008; Sharma et al. 2010).

The administration of hCG at the time of insemination allowed 87.23% of our mares to ovulate at 48 h. This ovulation rate is similar to that found by Berezowski et al. (2004) and McCue et al. (2007), and is well above the 69.6% found by Serres et al. (2010). However, values higher than 90% have been reported in different breeds (Blanchard et al. 2002; Davies Morel and Newcombe 2008; Cox et al. 2009).

The average time that elapsed until the mares were again in pregnancy was 28.34 days post-foaling. Further, a pregnancy rate at first oestrus of 39.48% was observed. This percentage is much smaller than those reported by Hevia et al. (1994), Nagy et al. (1998) and Sharma et al. (2010) from studs like Thoroughbreds, Trotters and Hungarian warmbloods.

In this work, mares delivering in winter became pregnant earlier than those delivering in summer or spring. These observations run counter to those of Nagy et al. (1998) and Sharma et al. (2010) who reported that mares foaling earlier in the year came into oestrus and ovulated later during the oestrous period.

The duration of gestation in equine species is highly variable (Perez et al. 2003; Heidler et al. 2004; Valera et al. 2006). In PRE, the different studies that have been carried out have reported values ranging from 304 to 372 days (Perez et al. 2003; Valera et al. 2006). Our data are consistent with these results, given that gestations of less than 305 days in duration resulted in immature, non-viable foals (Rossdale 1976).

We did not find any significant difference between the mares from the three different age groups in terms of the duration of pregnancy, which is in contrast to the observations recorded by the team of Valera et al. in 2006 in the same breed. These authors observed that the duration of gestation in these mares decreased with age, reaching a minimum at 10–12 years and then increasing slightly with age. Davies Morel et al. (2002) found no effect of maternal age on the duration of gestation but Demirci (1988) described a lengthening of the gestation process with increased maternal age. Concerning the effect of the gender of offspring, our study showed longer duration of pregnancy for colts than fillies, which is agreement with the observations of many other authors (Hevia et al. 1994; Davies Morel et al. 2002; Perez et al. 2003; Valera et al. 2006).

The pregnancy loss rate reported here is one of the lowest when compared to those reported previously (Ginther et al. 1985; Merkt and Jochle 1993; Nath et al. 2010; Sharma et al. 2010; Schulman et al. 2013). This may be explained by the reproductive

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management of the stud farm to reduce abortion cases. In our stud, mares were systematically vaccinated against equine herpesvirus type 1 and type 4 at five, seven and nine months of gestation in order to prevent cases of abortion. Also, these females were grouped in large pens according to their stage of pregnancy, in order to receive an equilibrated regimen of feed and to reduce physical accidents between mares. Finally, when mares foaled, each one received uterine lavage with saline solution at 3–4 days post-partum to remove all placenta and foetal leftover. Thus, infection was limited in the uterus before the mare was again inseminated during foal heat.

Our results showed also that pregnancy losses were highest for mares foaled in winter, those in lactation and during the first three months of conception. These losses could be due to the effect of season and insufficient uterine involution in the mares (Sharma et al. 2010).

In conclusion, in the PRE, the age, the category of mares and foaling season all influenced reproductive efficiency and pregnancy. Maidens and the youngest mares exhibited the smallest pre-ovulatory follicle sizes while females delivering in winter became pregnant earlier but for longer than mares delivering in other seasons. These findings could contribute to improving management strategies in "Pura Raza Española" mares.

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