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HORMONAL LEVELS AND FOLLICULAR DYNAMICS IN RELATION TO THE OESTROUS CYCLE IN BARB AND ARABIAN MARES, ALGERIA

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ABSTRACT

This current study is an effort to understand the hormonal and follicular growth in the Barb and Arabian mares during the oestrous cycle; as mares are unique creatures. A total of 53 mares with 97 oestrous cycles were studied. The mares with a mean age of 10.38 ± 4.55 were examined by ultrasonography every day during their breeding season (2017). Two blood samples from each mare (n = 24) were obtained for progesterone (P4), oestrogen (oestradiol-17 beta) and follicle-stimulating hormone (FSH) determinations. The data revealed that the duration of the oestrous cycle was between 19 to 22 days. The pre-ovulatory follicle grew (3.02 ± 1.04) millimetre per day. The rate of cycles exploited in the mare (Arabian versus Barb) for conception was significantly different (P < 0.001). The maximal diameter of the follicle was 50.00 millimetre. The serum progesterone levels (P < 0.01) in mares were significantly higher in the luteal phase than those recorded during the time of oestrous. However, the levels of oestradiol and for FSH did not significantly change during the oestrous cycle in the mares. Determining the association between the size of the follicle and the hormone profiles were the most reliable criterion in the prediction of ovulation.

Key words: Algeria; hormone; mare; oestrous cycle; ultrasonography

INTRODUCTION

For the successful breeding of horses, an understanding of the physiological changes in the reproductive status of mares is critical. The most important factors influencing the breeding performance of horses are generally: the stallion, the mare, the genetic variability and its relation to the semen characteristics, and the management practices of the animal husbandry [3, 19].

Breeding records exist in many forms (foaling rates, pregnancy rates per cycle, or per season rates), but there is still a need to develop tests that can predict fertility with a reasonable degree of certainty [18]. With such knowledge of the physiological status of mares, horse breeders can

better manage the breeding and husbandry of the animals [39].

Follicular dynamics is defined as the process of continual growth and regression of antral follicles [6, 33]. It is very important to understand the reproductive cycles of a mare. Mares are exceptional due to their capacity to have a considerable follicular growth during the oestrous and can even arrive during the luteal phase. Occasionally, an antral follicle reaches the pre-ovulatory size and ovulates during the dioestrus without any sign of heat, so the determination of the presence of a large follicle on an ovary is a poor predictor of oestrous in the mare [7].

Since the initial report of Palmer and Driancourt [27], ultrasonography has proven to be a very useful method for visualizing the reproductive tract of the mare [20], enabling the early diagnosis of pregnancy and the timing of ovulation and the study of follicular dynamics and embryo characteristics [37, 38]. Ultrasonography is also complementary to traditional methods used for the diagnosis of the female reproductive status, such as hormonal assays [1, 11, 30, 36]. The mare has been considered a relevant comparative research model for follicle studies because of a striking similarity with human females concerning their follicle dynamics and hormonal changes during the inter-ovulatory interval [14].

Several hormones have been demonstrated as potential factors that control the follicle growth and subordination. During the breeding season, the non-pregnant mare will have recurring oestrous cycles. The oestrous cycle is defined as the period from one ovulation to a subsequent ovulation, with each ovulation being accompanied by signs of oestrous and plasma progesterone concentrations below 1 $ng.ml^{-1}$ [12, 25]. The determination of the moment of the oestrous cycle during which the ovulatory follicle was recruited became an object of a debate. Follicles tend to grow by waves, at the rate of one or two waves by cycle [10, 15].

Mares are unique creatures. Mares show a different pattern of oestrous cycle and ovulation events when compared to other species. Therefore, understanding the physiological conditions and reproductive events of mares are necessary to improve their pregnancy rates [39]. The horse conceptus is unique in that it does not make stable contact with the uterine epithelium until 40—42 days after ovulation [4, 21].

The aim of this study was to monitor the follicular changes and predict the ovulation and determine the degree of correlation between follicular diameters measured before ovulation by ultrasonography and the serum concentrations of the three main hormones implicated in the oestrous cycle of mares. We describe then the strategies to estimate the moment of the ovulation during the oestrous cycle.

MATERIALS AND METHODS

The study area

This study took place at the National Haras of Tiaret which was created in 1877 on a surface of 800 ha. The province of Tiaret is characterized as a continental climate with harsh winters, and hot and dry summers. Currently, the National stud farm of Tiaret serves as the main supplier of horses while maintaining the model and the original type.

Data collection

Animals studied

This study was conducted during the breeding season between February and April 2017. Fifty-three mares with 97 oestrous cycles, were studied from Barb mares (n = 17) with 32 oestrous cycles and Arabian mares (n = 36), with 65 oestrous cycles. The mares were all of known fertility with no uterine pathology. The age of these females varied between 4 and 20 years, and their body condition ranged from 4 to 6 on a scale of 9 according to Henneke et al. [17].

Ultrasonography

The same operator performed the trans-rectal palpation and ultrasound examinations of the reproductive system, always, with portable ultrasound equipment using a 5 to 7 MHz multi-frequency linear probe (DRAMINSKI). The ultrasound examinations were performed daily or twice-daily on follicles ≥ 15 mm, described with details by Lemma et al. [23]. The mares were judged in oestrous if they presented: a doughy uterus, uterine folds, a dominant follicle ≥ 30 mm in diameter, and a soft cervix [28].

Hormonal analysis

Blood samples were obtained by jugular venepunctures using dry vacutainer tubes to collect two samples (n = 24) (over eight-day intervals during April 2017) with each ul-

trasound examination. Thimonier [35] reported that an interval (7 and 11 days) between the samples allowed the characterizing of the physiological state of all the females in the domestic species. The dosage of oestrogens (oestradiol-17 beta), of follicle-stimulating hormone (FSH) and the progesterone (P4) were determined through use of a solid-phase radioimmunoassay (RIA) using a commercial diagnostic kit from Immunotech®, in the laboratory of doctor Bellil (El-Khroub, Constantine).

Statistical analysis

The data collected were subjected to various statistical methods using SPSS 20 and expressed as the mean ± standard error (SD) min and max. Oestrous and luteal data were analysed for period effects using repeated measure analysis of variance (ANOVA), followed by the Student Newman–Keuls multiple and significance were set at $P < 0.05$ and $P < 0.01$. The Student Newman–Keuls multiple comparison test was also used to study the effect of age on follicular growth and hormone profiles. The Pearson's correlation coefficients were performed to assess the association between the parameters studied.

Table 1. Descriptive statistics of the reproductive cycle of mares during oestrous

	Mean	SD	Min	Max
Age	10.38	4.55	4.00	20.00
Oestrous cycle	20.43	0.85	19	22
Follicular growth	3.02	1.04	2	4
Number of cycles	1.84	0.93	1	5

Ethical statement

All the experiments conducted for this study were done only after obtaining the ethical approval from of National Haras of Chaouchoua Tiaret.

The authors declare that there is no conflict of interest.

RESULTS

The mean value and the standard deviation for the diameters of the follicles during the oestrous cycle, the follicular growth as well as the number of cycles exploited for conception are presented in Table 1 for all mares, both Arabian and Barb. The ultrasound examinations revealed that the duration of the oestrous cycle in Arabian mares were from 19 to 22 days. On the other hand, the mean diameters of the follicles during the oestrous cycle was 20.43 ± 0.85 millimetre with a daily growth of 2 to 4 millimetres (Fig. 1.).

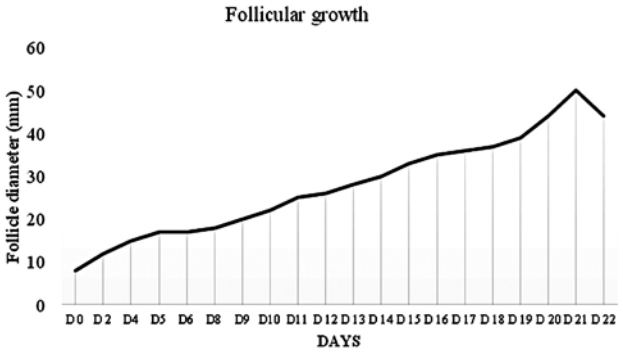

Fig. 1. Follicular dynamics of mare (aged 09 years).

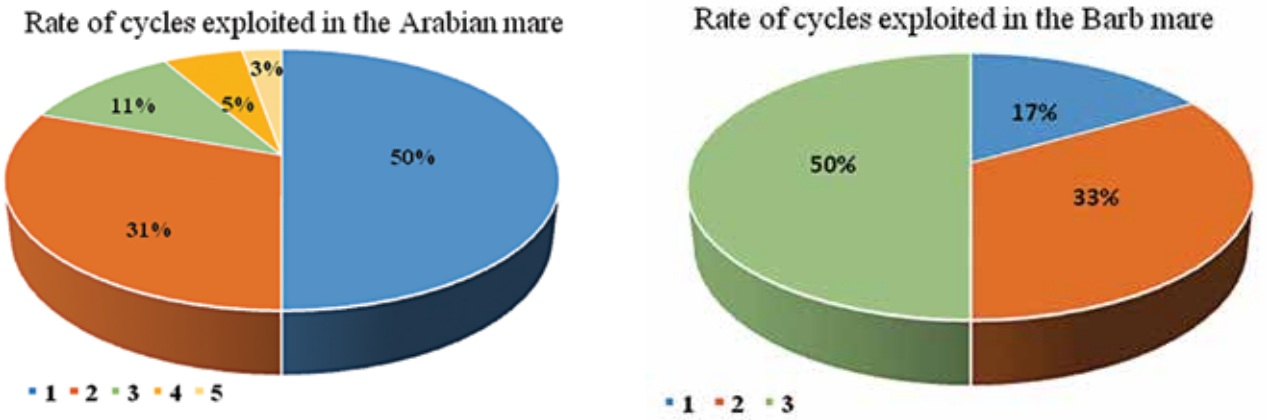

Fig. 2. Rate of cycles exploited in the mare (Arabian versus Barb) for conception ($P < 0.001$)

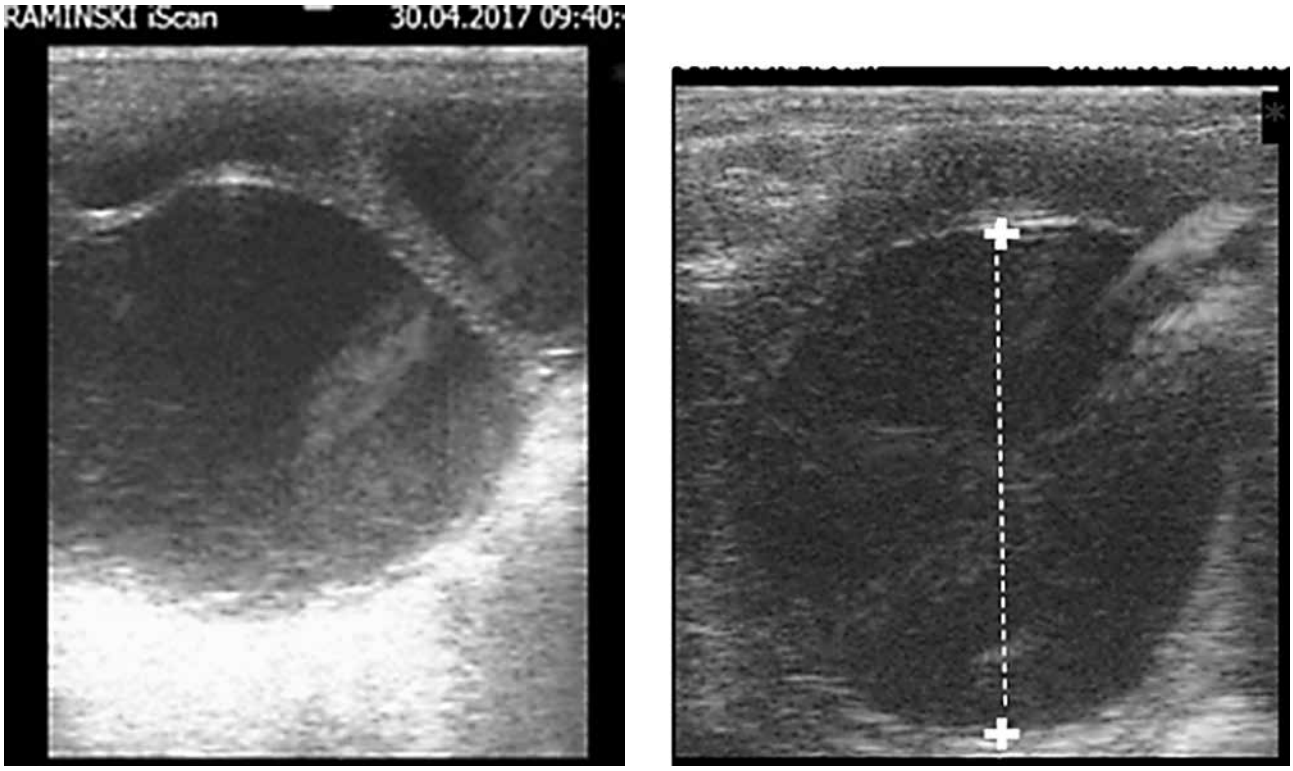

Fig. 3. The ultrasonographic echo of the dominant follicle

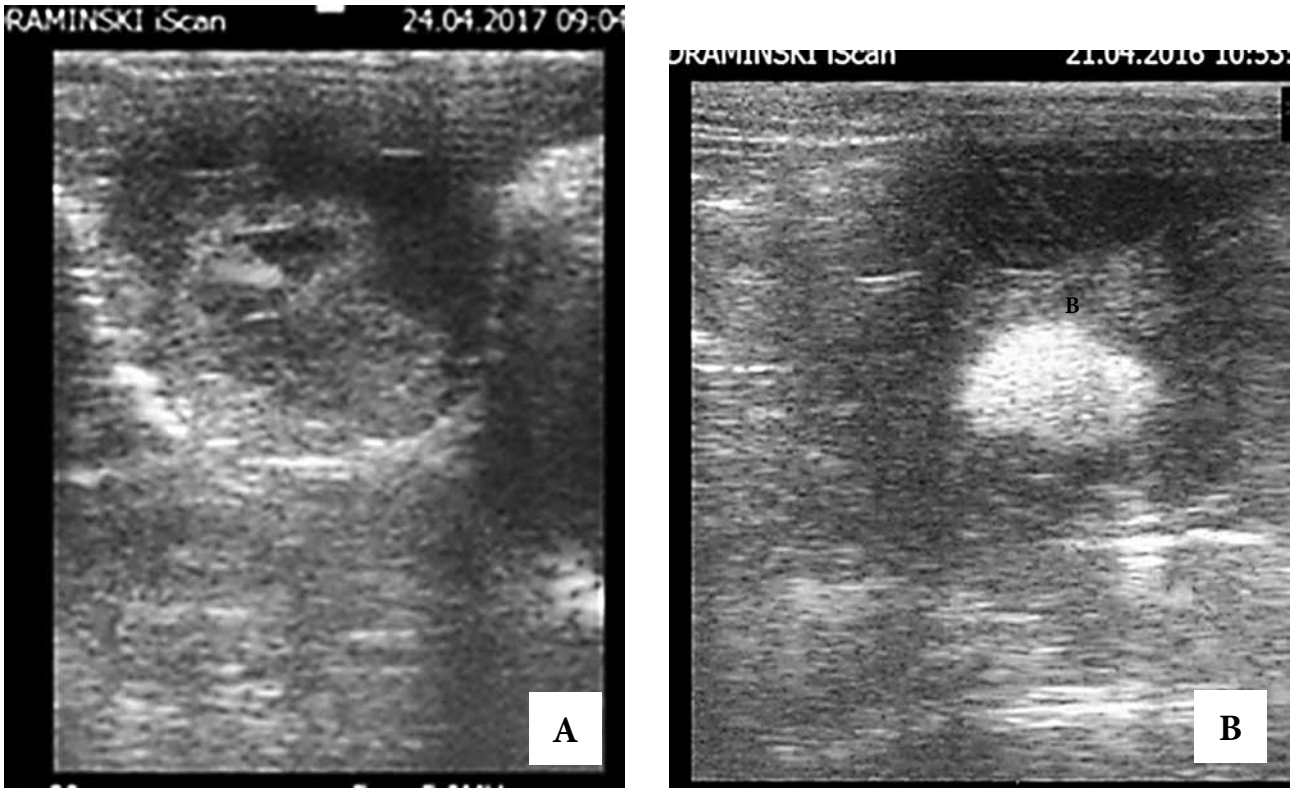

Fig. 4. Ultrasonographic images of corpus haemorrhage: recent ovulation (A); *corpus luteum* (B)

Table 2. Descriptive statistics of mares according the physiological stage

Variable	Physiological stage	Mean	SD	Min	Max	P value
Follicular diameter [mm]	E	31.47	14.54	15.00	50.00	0.036*
	D	27.00	14.94	15.00	47.00	
P4 [ng.ml^{-1}]	E	0.34	0.22	0.24	0.77	0.002**
	D	10.52	7.05	4.92	28.44	
Oestradiol [pmol.l^{-1}]	E	5.49	4.26	4.00	16.01	0.186
	D	7.36	2.45	4.00	9.00	
FSH [UI.L]	E	0.31	0.12	0.23	0.41	0.248
	D	0.29	0.21	0.21	0.37	

*—Correlation significant at P < 0.05; **—significant at P < 0.01; E—oestrous; D—dioestrus

Table 3. Coefficient of correlation among different parameter of mares during the oestrous

r	Age	Follicular diameter	P4 [ng.ml^{-1}]	Oestradiol [pmol.l^{-1}]	FSH [UI.l^{-1}]
Age	1				
Follicular diameter [mm]	–0.202	1			
P4 [ng.ml^{-1}]	0.396	0.49	1		
Oestradiol [pmol.l^{-1}]	0.139	0.06	–0.45	1	
FSH [UI.l^{-1}]	–0.230	–0.93**	–0.64	0.16	1

**—Correlation significant at P < 0.01

The number of oestrous cycles exploited for conception in the Arabian mares were five (50 % in the first cycle) versus three cycles exploited by the Barb mare (17 % in the first cycle), with a significant difference (P < 0.001) (Fig. 2.).

The descriptive statistics of the reproductive cycle of mares during oestrous is presented in Table 1.

The Figures 3 and 4 show respectively the ultrasonography of the dominant follicles and the *corpus luteum*.

The ultrasound examination demonstrated a significant difference in the growth of follicles between the mare in oestrous (15 to 50 mm) and dioestrus (15 to 47 mm) (P < 0.05) (Table 2, Fig. 3).

The serum progesterone levels (P4) in the mare, and follicular growth were significantly higher respectively (P < 0.01; P < 0.05) in the luteal phase of the oestrous cycle, then those recorded at the time of oestrous. However, the serum concentration of estradiol-17 beta and the FSH level did not significantly differ between oestrous and luteal phases of the mare oestrous cycle (P > 0.05) (Table 2).

The table 2 reveals the descriptive study of the hormonal and follicular parameters.

Table 3 shows a significant correlation between the level of FSH and the diameter of the follicular growth ($r = -0.93$; $P < 0.01$) during the oestrous cycle of mares. No difference was observed on the correlation among these parameters: follicular diameter, oestradiol-17 beta and P4.

DISCUSSION

This study demonstrated that the duration of the oestrous cycle of the mares were (19 to 22 days), with a follicular size between 15 and 50 mm. That was similar to the results reported by several other investigators [16, 24, 37, 39].

Warriach et al. [38] reported that the conception rate of the Arabian was 62 % in the first cycle mated in Pakistan; our results of a conception rate are different in the Arabian of 50 % and 17 % in the Barb. The higher conception rate in the Arabian mares may be due to a genetic trait of adaptability to warmer climates [38].

The ovulatory follicle grew at the rate of 2 to 4 mm per day, to arrive finally at the ovulatory stage; this is in agreement with the results found by others [5, 29, 30, 36]. Additionally, Blanchard et al. [7] reported that large follicles can be present during any stage of the oestrous cycle, so follicular size alone is not a reliable indicator of oestrous or dioestrus.

Mares in oestrous have an average level of the progesterone P4 0.34 ± 0.22 ng.ml^{-1}, during the oestrous, and (10.52 ± 7.05) during the dioestrus. The values of progesterone in this study are comparable to those reported by earlier authors [1, 2, 12, 22, 25, 26, 30].

The significant correlation of mares in oestrous were expressed between the rate of the FSH and the growth of the follicular size ($r = -0.93$; $P < 0.01$). The secretion of FSH was lower during the beginning of the oestrous because of the secretion of proteins inhibin-like products by the pre-ovulatory follicle, when the growing follicle reached the pre-ovulatory stage; it produced hormones, which inhibited the pituitary secretion of FSH [8, 9, 14, 24, 32, 34]. Furthermore, Tharasanit [34] reported that FSH declined when the size of the largest follicle reached approximately 13 mm.

The positive correlation between the follicular diameter and the progesterone P4 ($r = 0.49$; $P > 0.05$) was similar to the values reported by Tharasanit [34] when the follicle development remained during the elevated progesterone levels P4 [34]. A positive correlation between the FSH and the oestradiol level ($r = 0.16$; $P > 0.05$) was because of a synergic activity between the oestrogens and FSH by stimulation of the follicular growth [10]. A positive correlation between the growth of follicles and the rate of oestradiol was found ($r = 0.42$; $P > 0.05$). Numerous medium follicles can contribute to the increase of the concentration of oestrogens during the dioestrus; the *corpus luteum* can have a minor role in the increase of oestrogens during the dioestrus [10].

One study showed that no effect of mare age on oestradiol-17-beta serum, Ginther et al. [13]. Moreover, Rocha et al. [31] reported that, there was a lower oestradiol-17-beta serum concentration than the younger mares accompanied by smaller follicles at ovulation and a longer period from maximum follicle diameter to ovulation.

CONCLUSIONS

It was concluded that mares show a different pattern of oestrous cycle and ovulation, the presence of a large follicle by ultrasound on an ovary is a poor predictor of oestrous in the mare and large follicles can be present during any stage of the oestrous cycle, so follicular size alone is not a reliable indicator of oestrous or dioestrus. Consequently, the role of the hormone levels, especially the progesterone level, in the establishment of earlier pregnancy in the mare is very important. Finally, this study was taken to provide and to produce a guideline for veterinarians responsible for reproductive management of mares under Algerian conditions.

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