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Factors influencing the oestrus cycle of Arab mares

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This work aims to study the factors of variation of the oestrous cycle of Arab thoroughbred mares under the conditions of northern Tunisia. The duration of the oestrous and of the oestrus cycle of 182 mares naturally bred (NB, n=152) and undergoing artificial insemination (AI, n=30) were monitored daily from February to May during 2017 breeding campaign. The reproductive status of mares, their parity and the rank of the oestrous cycle were studied in function of the duration of the oestrous cycle and the oestrus. Results showed that the duration of the oestrous cycle was longer in maiden mares than in the lactating and in the barren ones ($p<0.05$). Moreover, this parameter was shorter starting from the 4th cycle for the mares conducted in NB ($p<0.05$), and longer during the 3rd cycle in AI mares ($p<0.05$). In addition, the duration of oestrus was shorter in older mares ($p<0.05$). It is interesting to notice that the oestrous cycle and oestrus durations decrease as the breeding season progresses ($p<0.05$). This work establishes, in conclusion, that the durations of the oestrous cycle and the oestrus in the Arab thoroughbred mares raised in northern Tunisia did not vary in the same way depending on the factors studied. Only reproductive status, cycle rank and the month of the breeding season affect the length of the oestrous cycle. In contrast, oestrus duration was affected only by the age of the mare and the month of the breeding season.

KEY WORDS: Arab mares / factors of variation / oestrous cycle / oestrus

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The mare is a female with a seasonal polyoestrous, regulated by the increasing length of the photoperiod, and is thus described as a long day breeder. Seasonality is controlled by extreme variations of the gonadotropins (FSH and LH) levels, and by oestrogen hormones [Alexander and Irvine 1991, Velez *et al.* 2012]. The current nomenclature states that the oestrous cycle has two clearly differentiated stages: i) oestrus or follicular phase lasting from 3 to 9 days, and ii) dioestrus or luteal phase lasting 14-15 days [Crowell-Davis 2007]. The observed variations in the oestrous cycle and oestrus durations are attributed to the daylight period variation, which depends on the geographical latitude of the animals location [Satué and Gardón 2013], and is mainly related to the secretion patterns of the melatonin during the dark period, determining the onsets of anoestrus and ovulation periods [Guillaume *et al* 1995]. It is well establish that the level of seric melatonin and the length of the brain's exposure influence the duration of the oestrous cycle during the transition periods [Donadeu and Ginther 2002]. Other authors attribute these variations to endogenous factors such as the breed [Tibary 1994] or the follicle dynamics [Driancourt *et al* 1983]. It should be noted that the studies focusing on the factors influencing the lengths of the oestrous cycle and oestrus are not numerous; and that most of them focus on the aspects affecting the fertility of mares [Bruck *et al* 1993, Benhajali *et al* 2010, Benhajali *et al* 2013, Lane *et al* 2016]. In fact, it is largely admitted that most mares ovulate about 24-48 hours prior the end of the oestrus, even though the mare's time ovulation is not well determined as for the ewe or the cow. Variations in the oestrus and the oestrous cycle durations made this determination more imprecise, which might lead to a loss of fertile cycles and thus, a decrease in pregnancy rate over the breeding campaign. Hence, several studies were conducted using managing tools such as exogenous hormones injections [Guillaume *et al* 2010, Henderson *et al* 2012], allowing horse breeders to manage reproduction more efficiently, and to improve mare's fertility.

In Tunisia, and even in North Africa, little or no studies were run in order to determine the particularities of the oestrus and the oestrus cycle durations, nor the factors influencing these parameters in the Arab mares. Furthermore, equine practitioners and breeders noticed the existence of variations in the oestrous cycle duration and the oestrus length, leading to the difficultly to determine the optimal moment for breeding/inseminating, and thus a lower conception rate, and a delay for the preparation of foals for national and international racing contests and other events.

For a better conception rate, and in order to identify the occurrence of the ovulation as precisely as possible, and so the success of the breeding season, this study was conducted to i) determine the duration of the oestrous cycle and the oestrus of Arab thoroughbred mares raised in northern Tunisia and free of any exogenous hormonal injection, and to ii) study the variation of these parameters according to the age, the reproductive status (lactating, maiden or barren), parity, rank of the cycle, and the month of the breeding season.

Material and methods

Study site

The current study was conducted in the National Foundation for the Improvement of the Horses Breed, located in Sidi Thabet, in the north of Tunisia, 24 km from the capital Tunis (36-54′31″ north, 10-02′33″ east). A total number of 182 Arab mares (average age = 10±4 years; age <15 years, n=160; age ≥15 years, n=22) were monitored during the breeding season from February to May 2017 (Tab. 1). The mares were assigned into 2 groups: 152 females led on natural breeding (NB), and 30 underwent an artificial insemination (AI). Weather and daylight duration during the experiment data were recorded each week amid the study period using the national meteorological station website (www.meteo.tn).

Table 1. Average variation of temperatures and daylight period during the breeding season

Item	February	March	April	May
Temperature (°C)	15.8±0.5	18±0.5	23±0.5	29±0.9
Daylight period (h)	11	12	13	14

Feeding

Mares were fed twice a day, according to their reproductive status; they received a ration (R1 or R2) to meet their nutritional needs. Lactating mares received R1, containing barley (27%), greenery (33%), hay (20%) and straw (20%), while the barren and the maiden ones received R2, consisting of barley (32.5%), greenery (35%), hay (14%) and straw (18.5%). During February, mash composed of barley, bran, linen, fava beans, corn, and a mineral vitamin supplement diluted in water and oil replaced barley in both rations.

Follicular ultrasonography

Oestrus detection in mares occurred daily through the bar test, in presence of a teaser. On the third day of the oestrus, a follow-up of the follicular activity in both ovaries was carried out, using an ultrasound scanner (Aloka 500 ®), equipped with a 5Mhz linear probe, in order to determine the diameter of the ovulatory follicle (in mm). This examination was practiced every 48 hours for the group of mares undergoing NB, and as soon as the ovulatory follicle reached 35 mm, the mare was mated every 48 hours until detection of the corpus luteum [Haras Nationaux 2004].

The frequency of ultrasonic examination was more consistent for the group of AI mares, for whom the ultrasonic scanning of the ovary carrying the growing follicle was practiced 3 times a day (9h, 16h and 23h) as soon as the follicle's diameter reached 35 mm, to monitor the appearance of the corpus luteum. When the corpus luteum was detected, we immediately performed the AI using frozen semen, by the deep post-ovulation method [Miro 2012, Najjar *et al* 2018].

For both groups and at each ultrasonic examination, the mare's parity and cycle's rank were recorded, whether she was naturally bred or underwent an AI, as well as her reproductive status (lactating, maiden or barren).

Mare's oestrous cycle

The duration of the oestrous cycle is defined by the time interval (days) between two successive ovulations, while the duration of the oestrus is the period (days) of oestrus behaviour expression in the mare, during one cycle [Bergfelt, 2000].

Statistical analysis

The recorded data were processed using the SAS software (SAS Institute Inc.®), and the variance analysis was performed with the GLM procedure, to study the effects of the way of reproduction (MR) – NB or AI, the mare's age (A), the month of breeding (BS), the reproductive status (RS), parity (P) and the cycle rank (CR) upon oestrous cycle and oestrus durations. Normality of the distribution of the oestrous cycle and oestrus were tested with the PROC plot, plot normal of the SAS package.

The statistical classification thus took the form of:

$$y_{ijklmn} = \mu + MR_i + A_j + BS_k + RS_l + P_m + CR_n + e_{ijklmn}$$

where

μ – overall mean;

e – random residual

The average comparison test was performed by the DUNCAN test and the significance level was set at p=0.05.

Results and discussion

The duration of the oestrous cycle (Fig. 1) did not vary between young and old mares, whether they were NB or AI ($p>0.05$). However, the duration of the oestrous behaviour was shorter in older mares (>15 years), in comparison to the younger ones, independently of the reproduction mode (5±0.9 and 6±0.6 vs. 8±1.0 and 9±0.3 days; Fig. 1, $p<0.05$). The average duration of the oestrous cycle was reported as 21 days [Bergfelt 2000] with extremes ranging from 18 to 21 days [Ginther *et al* 2008a]. Different authors link the variation in the cycle length to a metabolic change, particularly regarding the synthesis of estradiol and progesterone in the sexual organs [Aurich 2011, Satué and Gardón 2013]. In our study, the duration of the short oestrous cycle found in older mares contradicts the results reported by Ginther *et al* [2008b], signalling a longer interovulatory gap in mares older than 18 years. Moreover, in the same age class (>15 years), the follicular phase was characterized by weak follicular dynamics and a low growth rate of the ovulatory follicle. Claes *et al* [2017] reported the same findings than Ginther *et al* [2008b], with the difference that the growth rate of the ovulatory follicle was not affected by the age of the mare.

It was also established that the follicular dynamics and the length of the mare's oestrous cycle are influenced by the nycthemeron [Ginther *et al* 2004]. The length of the exposure to the darkness varies according to the latitude and to the season of the year, initiating transition periods between the anoestrus and the sexual seasons. Other factors, such as the mare's feeding and its body score, have positive influence on the mare's reproductive parameters [Ginther 1992, Gentry *et al* 2002].

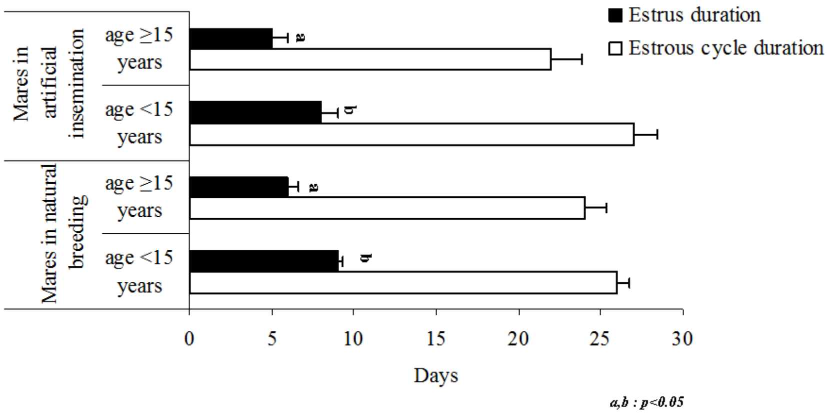

Fig. 1. Variation of the oestrous cycle and oestrus durations in relation to the mare's age (mean±se).

Regarding the reproductive status of the mares, the duration of the oestrous cycle was longer in maiden mares than in the lactating and the barren females (27 vs. 25 vs. 26 days, $p<0.05$). Besides, no influence of the reproductive status on the oestrus duration was noticed between the different groups of mares (age, breeding mode) (Fig. 2, $p>0.05$). Our results showed also that parity did not affect the duration of the oestrous cycle nor the oestrus length ($p>0.05$, Fig. 3).

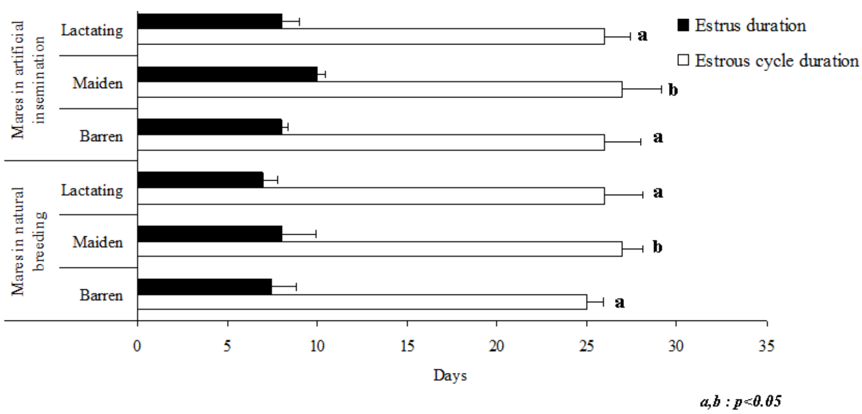

Fig. 2. Variation of the oestrous cycle and oestrus durations across mare reproductive status (mean±se).

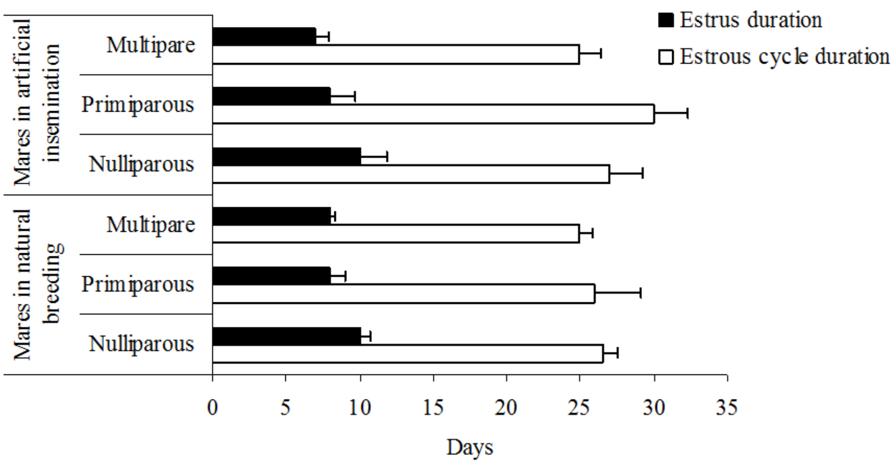

Fig. 3. Variation of the oestrous cycle and oestrus durations by mare parity (mean±se).

Data analysis showed also that the duration of the oestrous cycle decreases from February to May in the NB and AI mares (NB: 26 vs 23 days, $p<0.05$; AI:31 vs 21 days, $p<0.05$, Tab. 2), with a significantly shorter oestrus duration in the AI mares during the month of May, when compared to February (AI: 4 vs. 12 days, $p<0.05$, Tab. 2).

Table 2. Variation of the oestrous cycle and oestrus durations according to the month of breeding season (means±se)

Item	February	March	April	May
		mares bred naturally		
Estrous cycle duration (days)	26±1.1^{a}	26±1.0^{a}	27±1.4^{a}	23±1.9^{b}
Estrus duration (days)	8±0.7	9±0.6	9±0.5	7.5±0.9
		mares artificially inseminated		
Estrous cycle duration (days)	31±3.2^{a}	26±1.4^{a}	23±1.2^{b}	21±0.9^{b}
Estrus duration (days)	12±1.8^{a}	8±1.2^{a}	6±1.0^{ab}	4±0.7^{b}

^{ab}Within a row means bearing the same superscripts differ significantly at $p<0.05$.

Some authors report that the extreme variations in environmental temperatures affect the duration of oestrus and the moment of ovulation [Guerin and Wang 1994, Yoon 2012], while some others found no effect of the season (spring, summer) on these parameters [Van Vliet 2014]; besides, neither the reproductive status (barren, maiden, lactating) nor the age affected the interovulatory interval. Driancourt *et al.* [1983] reported no effect of the season on the follicular activity of pony mares during and outside the ovulatory season, as the follicular populations were similar in both periods, only the stage of preovulatory development is missing in pony mares during the anoestrus. In addition to those findings, sometimes contradictory, Matheu-Sánchez *et al.* [2016] recorded increased conception rate in mares exhibiting a longer oestrus. Moreover, Nagy *et al.* [1998] relate a longer foaling oestrus in primiparous mares than multiparous ones, with a tendency to an increased interovulatory interval, and no difference in the ovarian activity of lactating and non-lactating mares [Nagy *et al.* 1998]. The contradiction in all these results might be explained by the differences in latitudes at which the experiments were held, and by the inner specificities of the studied horse breeds.

In our study, the duration of the oestrous cycle varies also according to its rank. Indeed, this parameter is significantly shorter starting from the 4th cycle when the mare is led in natural breeding (21 days, $p<0.05$), while it is longer at the 3rd cycle of the AI mares (28 days, $p<0.05$). Unlike the duration of the oestrous cycle, the duration of the expression of the oestrus behaviour did not vary with the rank of the cycle (Fig. 4, $p>0.05$). Currently, no data studying the effect of the oestrus cycle's rank on the duration of the cycle and on the oestrus length in mares is available; the cycle's rank is solely linked to the conception rate [Warriach *et al.* 2014] and the mare fertility [Benhajali *et al.* 2010].

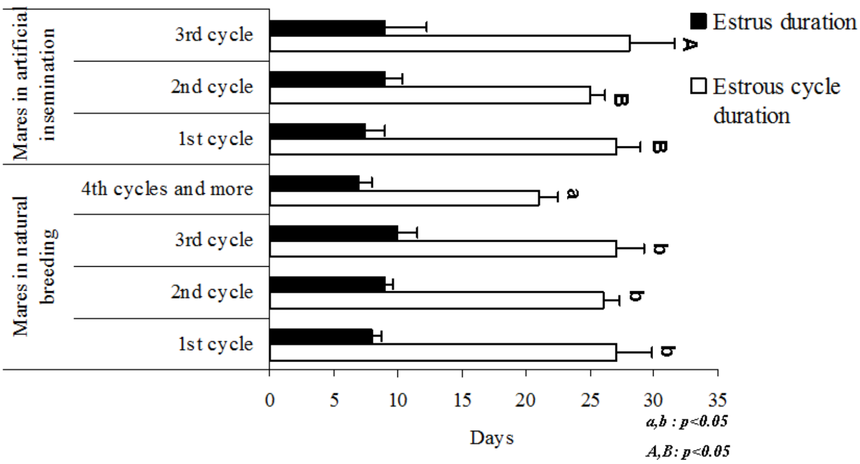

Fig. 4. Variation of the oestrous cycle and oestrus durations according to mare's rank of the cycle (mean±se).

In the same way, there are no studies describing how the way of reproduction (NB or AI) affects the oestrus duration in mare. Regarding this matter, our results showed no differences between the NB and AI mare groups for all the studied parameters. In our study, the results obtained on the Arab pure breed mares raised in Tunisia showed that the way of reproduction does not affect the oestrous cycle and the oestrus durations. It appears that practicing 3 ultrasonic follow-up determinations of the follicular activity in the AI mare group does not precise the durations nor the time of ovulation. For the other studied factors, the duration of the oestrous cycle and oestrus did not vary in the same way. Only the reproductive status, the cycle rank and the month of the breeding season affect the length of the oestrous cycle in the two groups of mares, while only the age of the mare and the month of the breeding season influence oestrus length. The limited number of observation for the AI group could in part explain this result, and it would be more interesting to verify the finding on a larger number of mares.

Any delay in conception would affect the birth date, and might lead to an inadequate management of the foal future career, hastening for instance the physical preparation and the training of racing horses. For this reason, and many others, such as work planning in the stud farm, determining as closely as possible the exact moment of ovulation is mandatory. When the ovulation occurs, the oocyte is able to survive for 24 hours, whilst the stallion's spermatozoa are able to remain up to 48-72 hours days in the female genital tract, versus 12-24 hours for the thawed ones [Ponthier *et al.* 2014]. Fecundation, and thus conception, is enabled when both gametes' survival intervals concurs. Male gametes delivered through AI have a shorter lifespan, hence the closer monitoring of the ovulation moment in the female, in order to make their presence successful, should coincide. The less sustained frequency of the ultrasonic examination in the naturally bred mare (once per 48 hours) is sufficient to estimate the moment of the ovulation of the tertiary follicle, and to submit her to the breeding service, especially if the stallion is highly priced and the number of matings allowed is restricted.

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REFERENCES

1. ALEXANDER SL, IRVINE CH., 1991 – Control of onset of breeding season in the mare and its artificial regulation by progesterone treatment. ***Journal of Reprodion and Fertility Supplement*** 44, 307-318.
2. AURICH C., 2011 – Reproductive cycles of horses. ***Animal Reproduction Science*** 124, 220-228.
3. BENHAJALI H, RICHARD-YRIS MA, EZZAOUIA M, CHARFI F, HAUSBERGER M., 2010 – Factors influencing conception rates of Arab mares in Tunisia. ***Animal Reproduction Science*** 117 (1-2), 106-110.
4. BENHAJALI H, EZZAOUIA M, LUNEL C, CHARFI F, Hausberger M., 2013 – Temporal Feeding Pattern May Influence Reproduction Efficiency, the Example of Breeding Mares. ***PLoS One*** 8(9), e73858.
5. BERGFELT DR., 2000. Estrous synchronization. In: Equine breeding management and artificial insemination. Samper, JC (ed.) Saunders Company, Philadelphia, pp165-177.
6. BRUCK I, ANDERSON GA, 1993. Hyland JH. Reproductive performance of Thoroughbred mares on six commercial stud farms. ***Australian Veterinary Journal*** 70, 299–303.
7. CLAES A, BALL BA, SCOGGIN KE, ROSER JF, WOODWARD EM, DAVOLLI GM, SQUIRES EL, TROEDSSON MHT., 2017. The influence of age, antral follicle count and diestrous ovulations on estrous cycle characteristics of mares. ***Theriogenology*** 97, 34-40.
8. CROWELL-DAVIS SL., 2007. Sexual behavior of mares. ***Hormones Behavior*** 52, 12-17.
9. DRIANCOURT MA, PRUNIER A, PALMER E, MARIANA JC., 1983. Seasonal effects on ovarian follicular devel-opment in pony mares. ***Reproduction Nutrition and Developement*** 23 (2A), 207-215.

10. DONADEU FX, GINTHER OJ, 2002. Changes in concentration of follicular fluid factors during follicule selection in mares. ***Biology of Reproduction*** 66, 1111-1118.
11. GENTRY LR, THOMPSON DL, GENTRY GT, DAVIS KA, GODKE RA, CARTMILL, JÁ., 2002. The relationship between body condition, leptin, and reproductive and hormonal characteristics of mares during the seasonal anovulatory period. ***Journal of Animal Science*** 80, 2695-2703.
12. GINTHER OJ., 1992. Reproductive biology of the mare: Basic and applied aspects. 2.ed. Cross Plains, WI, USA: Equiservices Publishing, pp105-172.
13. GINTHER OJ, GASTAL EL, GASTAL MO, BEG MA., 2004. Seasonal influence on equine follicle dynamics. ***Animal Reproduction*** 1 (1), 31-44.
14. GINTHER OJ, BEG MA, NEVES AP, MATTOS RC, PETRUCCI BP, GASTAL MO, GASTAL EL., 2008a. Miniature ponies: 2. Endocrinology of the oestrous cycle. ***Reproduction Fertility and Developement*** 20, 386-390.
15. GINTHER OJ, GASTAL MO, GASTAL EL, JACOB JC, SIDDIQUI MA, BEG MA., 2008b. Effects of age on follicle and hormone dynamics during the oestrous cycle in mares. ***Reproduction Fertility and Developement*** 20(8), 955-63.
16. GUERIN MV, WANG XJ., 1994. Environmental temperature has an influence on timing of the first ovulation of seasonal estrus in the mare. ***Theriogenology*** 42(6), 1053-1060.
17. GUILLAUME D, RIO N, TOUTAIN PL, 1995. Kenetic studies and production rate of melatonin in poney mare. ***American Journal of Physiology and Regulatory Components of Physiology*** 268, 1236-1241.
18. GUILLAUME D, SALAZAR-ORTIZ J, MENASSOL JP, MALPAUX B, CHEMINEAU P, 2010. Photoperiod, metabolism and reproduction : Advantage of the equin model. *Bulletin* ***Académie Vétérinaire de France*** 163(1), 5-18.
19. HARAS NATIONAUX, 2004. Insémination artificielle équine. Guide pratique. 3rd edition, edited by Les Haras Nationaux, Direction des connaissances, ENPH, 61310 Le Pin au Haras.
20. HENDERSON ISF, BRAMA P, OSBORNE P, BELTMAN ME, 2012. Interovulatory intervals in mares receiving Deslorelin implants in Irland (2009 to 2010). ***Veterinary Record*** 1-5.
21. LANE EA, BIJNEN MLJ, OSBORNE M, MORE SJ, HENDERSON ISF, DUFFY P, CROWE MA. , 2016. Key Factors Affecting Reproductive Success of Thoroughbred Mares and Stallions on a Commercial Stud Farm. ***Reproduction in Domestic Animal*** 51(2), 181-187.
22. MATEU-SÁNCHEZ S, NEWCOMBE JR, GARCÉS-NARRO C, CUERVO-ARANGO J., 2016. The period of the follicular phase during which the uterus of mares shows estrus-like echotexture influences the subsequent pregnancy rate. ***Theriogenology*** 86(6), 1506-1515.
23. MIRO J. , 2012. Ovarian ultrasonography in the mare. ***Reproduction in Domestic Animals*** 47 Suppl 3, 30-3.
24. NAGY P, HUSZENICZA GY, JUHÁSZ J, KULCSÁR M, SOLTI L, REICZIGEL J, ABAVÁRY K., 1998. Factors influencing ovarian activity and sexual behavior of postpartum mares under farm conditions. ***Theriogenology*** 50(7), 1109-1119.
25. NAJJAR A, KHALDI S, BEN SAID S, HAMROUNI A, BENAOUN B, EZZAOUIA M., 2018. Variation factors of the pregnancy rate of Arab pure Breed mares inseminated by the deep intracornual method in post-ovulation. ***Advances in Animal and Veterinary Science*** 6(1), 40-43.
26. PONTHIER J, VAN DEN BERGHE F, PARRILLA HERNANDEZ S, HANZEN C, DELEUZE S., 2014. Congélation de sperme dans l'espèce équine: état des lieux et perspectives. ***Annales de Médecine Vétérinaire*** Université de Liège, 55-71.
27. SATUÉ K, GARDÓN JC., 2013. A Review of the Estrous Cycle and the Neuroendocrine Mechanisms in the Mare. ***Journal of Steroids and Hormonal Science*** 4, 115.
28. TIBARY A., 1994. Reproduction equine, Tome 1: La jument. Actes Editions.

29. VELEZ IC, PACK JD, PORTER MB, SHARP DC, AMSTALDEN M, WILIAMS GL., 2012. Secretion of luteinizing hormone into pituitary venous effluent of the follicular and luteal phase mare: novel acceleration of episodic release during constant infusion of gonadotropin-releasing hormone. ***Domestic Animal Endocrinology*** 42, 121-128.
30. VAN VLIET D., 2014. The oestrous cycle in Friesian mares. Master thesis. Faculty of Veterinary Medicine. Utrecht University.
31. YOON M., 2012. The Estrous Cycle and Induction of Ovulation in Mare. ***Journal of Animal Science and Technology*** 54(3), 165-174.
32. WARRIACH HM, MEMON MA, AHMAD N, NORMAN ST, GHAFAR A, ARIF M., 2014. Reproductive Performance of Arabian and Thoroughbred Mares under Subtropical Conditions of Pakistan. ***Asian-Australasian Journal of Animal Science*** 27(7), 932-936.