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The effect of ovarian laterality on various reproductive parameters in Arabian mares

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ABSTRACT

Objective: The present study aimed to investigate the effect of ovarian laterality on pregnancy outcomes and foal sex and to explore the role of age and parity in determining the ovulation side in Arabian mares.

Materials and Methods: Data were collected from 358 mares over a five-year period, during which 885 estrus cycles were monitored. The side of ovulation was determined using ultrasonography, and pregnancy was confirmed on days 14, 28, and 45 post ovulations. The mares were categorized into four age groups as 3-8, 9-13, 14-17, and ≥ 18 years.

Results: The laterality of ovulation was evenly distributed between the right (49.3%) and left (50.7%) ovaries, with no significant difference in pregnancy outcomes between the two sides. The majority of pregnancies resulted from the first ovulation (51.9%), and pregnancy outcomes did not differ significantly among the ovulatory sequences. Likewise, no significant relationship was found between the ovulation side and foal sex. The distribution of ovulation sides was similar across the age groups; however, a significant relationship was observed between the age group and the ovulation side that resulted in pregnancy ($p<0.05$), with a general decline in pregnancies observed between the younger and older age groups on both sides of ovulation. Younger mares had higher pregnancy rates, with left-side ovulation rates of 50.0% and 41.1% for mares aged 3–8 and 9–13 years, respectively, compared to 13.9% and 5.3% for mares aged 14–17 years and 18 years and older. Similarly, right-side ovulation rates were 43.7% and 33.5% for younger mares, which were higher than the rates of 11.0% and 1.3% for older mares. Additionally, the parous state of the mares did not significantly influence the side of ovulation or pregnancy outcomes.

Conclusion: Overall, in Arabian mares, ovarian laterality does not significantly influence pregnancy outcomes or foal sex and is not affected by parity or age, although a decrease in pregnancy rates with increasing mare age was evident. These results provide insights into the dynamics of ovarian laterality in Arabian mares and their impact on reproductive efficiency, which can guide the development of breeding strategies and assisted reproductive techniques.

Keywords: *Age, Equine, Foal sex, Ovulation side, Parity, Pregnancy*

INTRODUCTION

In many species, the laterality of reproduction holds significant biological and clinical importance in reproductive studies and management, as this phenomenon influences reproductive efficiency, embryo development, and the success of assisted reproductive techniques. While some species exhibit anatomical asymmetry between the right and left sides, others have functional inequality. For instance, in llamas and alpacas, gestation and

embryo implantation occur solely in the left uterine horn, irrespective of the ovulation side, highlighting a significant asymmetry in which the left horn is larger than the right horn (Sumar and Adams, 2007; Ratto et al., 2020). The dominance of the left ovary is well documented in many avian species, as the right ovary typically remains underdeveloped and nonfunctional (Delehanty and O'Hearn, 2005; Wan et al., 2017; Peng et al., 2023).

In addition to these anatomical asymmetries, ovarian function may differ significantly between left and right ovaries. In golden hamsters, a right-side biased asymmetry in the number of corpora lutea has been noted, regardless of housing conditions (Chow and Chow, 1987; Fritzsche et al., 2000). Similarly, the predominance of the right ovary in producing more corpus luteum and harboring a greater quantity of larger follicles has been observed in several ruminant species, including sheep (Shabankareh et al., 2009) and goats (Grizelj et al., 2013), with similar patterns reported for cattle ovulation frequency (Cushman et al., 2005; Karamishabankareh et al., 2015). Furthermore, in humans, the right ovary demonstrates superior performance compared with the left ovary, exhibiting a higher rate of ovulation, producing oocytes with greater pregnancy potential, and showing better follicle recruitment and oocyte retrieval during in vitro fertilization. This pattern is consistently observed in both fertile and infertile women with healthy ovaries (Fukuda et al., 2000; Lan et al., 2010). However, some studies have suggested an equal distribution of ovulation between the two ovaries (Balasch et al., 1994; Fukuda et al., 1996). Contrasting evidence challenges the categorization of a species into a specific ovulation pattern, as evidenced by research on mares, which reported symmetrical (random) as well as asymmetrical patterns of ovulation favoring the left ovary (Wesson and Ginther, 1981; Sanderson, 1982; Ginther, 1983).

Studies on reproductive laterality have gained increasing interest because of their potential influence on sex-ratio determination. The uneven sex distribution observed in the uterus of cattle, gerbils, rabbits, and mice implies a consistent asymmetry in either the ovaries or uterine horns, which influences offspring sex distribution (Pearson, 1949; Wimsatt, 1975; Younglai et al., 1981; Baird and Birney, 1985; Endo et al., 1987; Hylan et al., 2009). Experimental surgical translocation in Mongolian gerbils has demonstrated that sex ratio inversion is primarily determined by the ovary of origin rather than the uterine environment, suggesting a mechanism involving biased selection of X- or Y-bearing sperm during fertilization (Clark et al., 1994). Comparable mechanisms have been identified in livestock (Grant and Chamley, 2007), with additional contributing factors including testosterone concentrations in follicular fluid (Grant and Irwin, 2005; Grant et al., 2008), insemination timing (Wehner et al., 1997; Martinez et al., 2004), and the maturation state of the oocyte (Dominko and First, 1997; Gutierrez-Adan, 1999). Furthermore, sex distribution appears to be breed-dependent, as studies have reported an asymmetric male-female distribution between uterine horns in beef cattle (Hylan et al., 2009; Giraldo et al., 2010), whereas Holstein dairy cows exhibit a more balanced distribution (Gharagozlou et al., 2013).

In the equine industry, where the ability to predetermine foal sex has significant economic and breeding implications, maternal factors, including age, body condition, and parity, have been closely linked to offspring sex (Cameron et al., 1999; Rezagholizadeh et al., 2015; Santos et al., 2015; Hall et al., 2022). For instance, mares under 15 years of age are more likely to produce male foals, whereas maiden and older mares tend to deliver female offspring, possibly due to mechanisms related to mitochondrial DNA and endometrial function (Kuhl et al., 2015; Flores, 2024). In addition, the Trivers-Willard hypothesis suggests that mares in better body condition at conception are more likely to produce male offspring. Other factors such as natural mating, gestation length, and genetic lineage have also been identified as potential factors influencing foal sex (Gutiérrez-Adán et al., 1999; Martinez et al., 2004; Cameron and Linklater, 2007; Hylan et al., 2009; Giraldo et al., 2010; Santos et al., 2015). Apart from these research, in a recent study conducted on Thoroughbred horses, left-sided ovulations resulted in a significantly higher proportion of male foals than right-sided ovulations (Rezagholizadeh et al., 2015). Collectively, these studies emphasize the relationship between reproductive laterality and sex determination, highlighting the need for further research on the underlying mechanisms of lateral asymmetry in this field.

Understanding the dynamics of asymmetry in ovarian structures and its influence on factors such as ovulation frequency, pregnancy outcomes, and potential offspring sex ratios is crucial for enhancing reproductive outcomes in both natural and assisted reproduction. However, in equines,

conflicting reports have challenged the categorization of mares into specific ovulatory patterns. In addition, to date, there has been a lack of research investigating the relationship between the ovulation side and foal sex distribution in Arabian mares, highlighting a significant gap in our understanding of reproductive laterality in this specific breed. Thus, this study aimed to investigate the effect of ovarian laterality on various reproductive parameters in Arabian mares, including pregnancy outcomes, foal sex, and the influence of age and parity on these parameters. Through the analysis of these aspects, this research sought to expand the knowledge base regarding ovarian functions in mares and provide valuable insights that could potentially inform breeding strategies and assisted reproductive techniques in the equine industry.

MATERIALS and METHODS

Animals and data collection

The study was conducted during the breeding season (February-June) over a five-year period, involving data obtained from 358 Arabian mares, which were monitored 546 times across various breeding seasons. The mares, housed individually at a Stud Farm in Eskişehir, Türkiye, were provided with alfalfa hay and commercial concentrate and had unlimited access to water. Only mares with no prior general health or gynecological issues were included. The mares, aged between 3 and 24 years, with previous offspring between 0 (maiden) and 14 years, were mated with a total of 46 stallions. In total, 885 estrus cycles (up to the 6th consecutive ovulation according to the timing within the breeding season) were monitored using ultrasonography with a 5–10 MHz linear endorectal L52x probe (Sonosite II, Fujifilm, Japan. The decision to breed mares was based on the presence of a preovulatory follicle with minimum diameter of 40 mm, the degree of uterine edema, cervical tone, and intrauterine fluid accumulation (Scarlet et al., 2023). The ovulation side was determined based on the location of the emerging corpus luteum following ovulation. Unilateral or bilateral double ovulations were excluded from the study. Pregnancy was determined ultrasonographically on the 14th day following ovulation and confirmed on days 28th and 45th days post-ovulation. Mares who did not maintain their pregnancies after the initial determination were excluded from the study. The sex of foals was recorded after birth. According to their age during the breeding season that they were monitored, mares were divided into four classes (Scoggin, 2015): 3-8 years old (n=243), 9-13 years old (n=206), 14-17 years old (n=74), and ≥18 years old (n=23).

Statistical analyses

Descriptive statistics were calculated for the data, and considering the distributions of the data, percentages or "Median (Minimum-Maximum)" were provided. Statistical analysis was performed to evaluate differences in the proportions of the side of ovulation based on pregnancy outcome, ovulation sequence, foal sex, age group, and parity variables, as well as to evaluate the significance of differences in the proportions of the side of ovulation leading to pregnancy according to age group and parity variables using the chi-square test. Additionally, the chi-square test was used to evaluate the statistical significance of differences in the proportions of ovulatory sequences according to pregnancy status. As the assumptions for parametric tests were not met, the Mann-Whitney U test was employed to evaluate the statistical significance of differences in the side of ovulation and the side of ovulation resulting in pregnancy according to parity. A significance level of $p<0.05$ was set for all statistical analyses. Data were analyzed using the SPSS 27 software package.

Ethical approval

This study was conducted in accordance with the decision of the Local Ethics Committee of Animal Experiments at Eskisehir Osmangazi University, dated 20.05.2024, and numbered 108.

RESULTS

The laterality of all ovulations (n=885) was evenly distributed between right (n= 436, 49.3%) and left (n = 449, 50.7%) ovaries. Likewise, the sides of the ovulations that resulted in pregnancy were similar between the right (n=244, 51.4%) and left ovaries (n=231, 48.6%). There were no significant differences between the right and left ovulations in terms of pregnancy outcomes (Table 1).

Table 1. Pregnancy outcome regarding the side of the ovulation.

Ovulation side	Pregnancy (%)		P
	Non-pregnant	Pregnant	
Left ovary	219 (48.8)	231 (51.2)	0.138
Right ovary	191 (43.8)	244 (56.2)	

The percentage of non-pregnant animals was 12.8%, and the majority of pregnancies resulted from the first ovulation (51.9%), followed by the second (22.9%), third (9.5%), and fourth to sixth ovulations (2.8%) of the breeding seasons. When all ovulations were considered, the pregnancy outcomes of consecutive ovulations did not differ significantly among the ovulatory sequences (Table 2). Similarly, there were no significant differences in the distribution of the ovulation side related to consecutive ovulations within a breeding season. The side of consecutive ovulations within a season was the same in 89 (38.7%) mares.

Table 2. Overall pregnancy outcome and the side of the ovulation regarding the ovulatory sequence.

Ovulatory sequence	Ovulation side (%)		p	Pregnancy (%)		p
	Left ovary	Right ovary		Non-pregnant	Pregnant	
1st Ovulation	262 (48.0)	284 (52.0)	0.117	263 (48.2)	283 (51.8)	0.253
2nd Ovulation	132 (57.4)	98 (42.6)		106 (46.1)	125 (53.9)	
3rd Ovulation	42 (49.4)	43 (50.6)		32 (37.6)	52 (62.4)	
4-5-6th Ovulations	13 (54.2)	11 (45.8)		9 (37.5)	15 (62.5)	

Over the course of five breeding seasons, 230 male and 245 female foals were born. However, no significant relationship was found between the ovulation side and foal sex (Table 3).

Table 3. Foal sex regarding the side of the ovulation.

Ovulation side	Foal sex (%)		P
	Female	Male	
Left ovary	115 (49.8)	116 (50.2)	0.446
Right ovary	130 (53.3)	114 (46.7)	

Mares were divided into four age groups to investigate the effects of mare age. While the majority of the overall ovulations (n=700, 79.1%) were from younger mares aged–3-13 years old, 20.9% of the ovulations were from older mares aged 14–24 years. The distribution of the sides of all ovulations was similar between the age groups (Table 4).

Table 4. The side of the ovulation regarding the different age groups of the mares.

Age groups	Ovulation side (%)		p
	Left ovary	Right ovary	
3-8	180 (48.9)	188 (51.1)	0.200
9-13	176 (53.0)	156 (47.0)	
14-17	79 (53.7)	68 (46.3)	
≥18	14 (36.8)	24 (63.2)	

The distribution of pregnancies was 46.9%, 37.2%, 12.4%, and 3.4% among age groups, respectively. A general decline in the number of pregnancies was observed between the younger and older age groups on both sides of ovulation. While the younger group aged between 3 and 8 years had a 60.5% (223/368) successful pregnancy rate regarding the number of all ovulations, it was 53.3% (177/332) for the 9-13 years of age group, followed by 40.1% (59/147) and 42.1% (16/38) for the 14-17 years old and ≥18 years old age groups, respectively. There was a significant relationship between the age groups and ovulation side that resulted in pregnancy (Table 5).

Table 5. The side of the ovulation resulted in pregnancy regarding the different age groups of the mares.

Age groups	Ovulation side (%)		p
	Left ovary	Right ovary	
3-8	101[a] (43.7)	122[a] (50.0)	0.026
9-13	95[a] (41.1)	82[a] (33.5)	
14-17	32[b] (13.9)	27[b] (11.0)	
≥18	3[b] (1.3)	13[b] (5.3)	

a,b: Different letters in the same column indicate statistically significant differences ($p<0.05$)

The rate of ovulations resulting in pregnancy was significantly higher in younger mares aged 3-8 years and 9-13 years (43.7%-41.1%) compared to mares aged 14-17 years and those 18 years and older (13.9%-1.3%) for left side ovulations ($p<0.05$). A similar pattern was observed for right side ovulations as well, with pregnancy rates being higher in mares aged 3-8 years and 9-13 years

(50.0%-33.5%) compared to mares aged 14-17 years and those 18 years and older (11.0%-5.3%) ($p<0.05$).

There was no significant relationship between the side of ovulation and the parous state of the mares. In both maiden and parous animals, the sides of all ovulations, as well as the side of ovulation that resulted in pregnancy, were similar between the right and left ovaries (Table 6).

Table 6. The side of the ovulations regarding the parous state of the mares.

Parous state	Ovulation side (%)		p	Ovulation side resulted in pregnancy (%)		p
	Left ovary	Right ovary		Left ovary	Right ovary	
Maiden	68 (47.6)	75 (52.4)	0.406	42 (45.7)	50 (54.3)	0.524
Parous	381 (51.3)	361 (48.7)		189 (49.3)	194 (50.7)	

The parity of the mares did not differ significantly with respect to the side of all ovulations or the ovulation side that resulted in pregnancy (Table 7). The median parity was 3 for both sides of the ovulations when all ovulations were considered.

Table 7. The parity of the mares regarding the ovulation side resulted in pregnancy and the side of all ovulations.

		Parity		p
		N	Median (Min.-Max.)	
Ovulation side resulted in pregnancy	Left	231	3 (0-10)	0.474
	Right	244	2 (0-13)	
Ovulation side	Left	449	3 (0-14)	0.649
	Right	436	3 (0-14)	

DISCUSSION

Understanding ovulation laterality is crucial for optimizing breeding strategies, particularly when using techniques such as artificial insemination or embryo transfer, as it can guide veterinarians in determining the most appropriate timing and method for insemination, ensuring that sperm and embryos effectively navigate the oviduct and uterine environment and may also influence the choice of the ovary for follicular aspiration during assisted reproduction. The current study revealed a balanced distribution of ovulation between the right and left ovaries in mares, aligning with earlier equine studies (Bain and Howey, 1975; Sanderson, 1982; Butterfield and Mathews, 1979) as well as with recent research on Thoroughbred mares, in which Rezagholizadeh et al. (2015) reported 51.3% left and 48.7% right ovulations, while Morel and O'Sullivan (2001) observed 50.6% left and 49.4% right ovulations, with no statistically significant differences ($p>0.05$), suggesting that both ovaries in mares have equal functionality, supporting their classification as symmetrical ovulators. Challenging these studies, Najjar et al. (2018) investigated the impact of the ovulation site on pregnancy rates in 62 Pure Arab Breed mares inseminated with frozen semen using the deep intracornual method post-ovulation and showed a significant difference in pregnancy rates based on the ovulation site, with mares ovulating from the right ovary achieving a 59% pregnancy rate compared to only 23% for those ovulating from the left ovary. The contrasting results might be attributed to the limited population size of the study or methodological differences, such as the use of artificial insemination with frozen semen, whereas in the present study, the mares were bred under natural cover.

The complex interplay among mare age, ovulation patterns, and fertility outcomes has significant implications for equine reproductive management and breeding strategies. The current observation that the incidence of left and right ovulations did not differ significantly among age groups is consistent with the results reported by Morel and O'Sullivan (2001), who conducted a comprehensive study on 828 mares across six age groups (3-5, 6-8, 9-11, 12-14, 15-18, and 18-22 years) over three consecutive years. This consistency was further supported by Rezagholizadeh et al. (2015), who also found that age did not influence the incidence of ovulation in the left and right ovaries. However, the current study highlights a general decline in the number of pregnancies between the younger and older age groups, despite a similar distribution of ovulation. This suggests that, while the ovulation pattern remains consistent across age groups, other age-related factors may influence fertility outcomes. An examination of existing research consistently reveals that a mare's reproductive efficiency is

primarily influenced by age, establishing it as one of the most reliable determinants in this field. Studies categorizing mares into age brackets (2-8, 9-13, 14-18, and >18 years) comparable to the current investigation revealed that early stage (days 15-21) pregnancy rates per cycle typically began to decline when mares reached 14 years of age. Notably, the day 40-42 pregnancy rates per cycle in both the United States and the United Kingdom showed signs of a decrease at an even earlier age. These results demonstrate a consistent downward trend in pregnancy rates across successive age groups (Allen et al., 2007; Bosh et al., 2009; Nath, 2010). The relationship between increasing maternal age and declining fertility is complex and requires further investigation, as it is influenced by multiple factors, including reduced viability of oocytes and embryos, deterioration of reproductive structures, and alterations in fetal membrane development, which are not independent of each other and can occur simultaneously, leading to decreased reproductive efficiency across different measures (Scoggin, 2015).

In the current study, no significant differences were observed in pregnancy outcomes among consecutive ovulations or between left- and right-sided ovulations. Notably, 38.7% of the mares consistently ovulated from the same side within a breeding season. There was no literature investigating the relationship among ovulatory sequence within the breeding season, the side of the ovulation, and pregnancy outcome in mares, however, Morel and O'Sullivan 2001 reported a statistically significant ($p<0.05$) higher likelihood that successive ovulatory cycles would ovulate on alternating ovaries rather than on the same ovary. Similarly, research focusing on female patients undergoing infertility treatment has revealed that ovulation patterns across consecutive cycles can significantly affect pregnancy outcomes in both intrauterine insemination and in vitro fertilization treatments. Contralateral ovulation, in which ovulation alternates between left and right ovaries in consecutive cycles, appears to have better outcomes than ipsilateral ovulation (ovulation from the same ovary in consecutive cycles). In particular, the best pregnancy outcomes were observed when ovulation occurred in the right ovary following left ovulation in the previous cycle (contralateral right-sided ovulation) (Fukuda et al., 2006). These findings emphasize the need for further research to explore the relationship between ovulatory sequence and ovulation side in mares.

The relationship between the ovulation side and offspring sex has been a subject of interest in various mammalian species, with conflicting results reported across different breeds and species. In the current study conducted on Arabian mares, 230 male (48.4%) and 245 female foals (51.6%) were born over five breeding seasons, which is comparable to the male foal ratio (46.1%-46.6%) previously reported for the Thoroughbred breed (Gharagozlou et al., 2014; Rezagholizadeh et al., 2014). However, in the present study, there was no significant relationship between the ovulation side and foal sex. This finding contrasts with a recent study on Thoroughbred horses, which reported a significantly higher proportion of male foals resulting from left-sided (57.4%) than from right-sided (35.3%) ovulations. Additionally, right-sided ovulation produced a lower male foal ratio than expected, whereas left-sided ovulation and overall male foal ratios did not differ significantly from the expected 50%. ($p=0.026$). This discrepancy in the results highlights the potential for breed-specific differences in reproductive laterality and its influence on offspring sex ratios. Interestingly, similar species-specific differences have been observed in cattle as well. Research in beef cattle has unveiled an asymmetrical distribution of offspring sex, with right-sided ovulations having a higher likelihood of male offspring, whereas left-sided ovulations were more likely to result in female calves (Hylan et al., 2009; Giraldo et al., 2010), although, in Holstein dairy cows, a parallel distribution of offspring sex was observed between the left and right ovulations (Gharagozlou et al., 2013).

In addition to these patterns, the relationship between ovarian laterality and parity in mares has been a subject of debate in equine reproductive research, with conflicting findings reported in the literature. Ginther (1983) utilized theriogenology reports of a mixed-bred population of mares consisting mainly of Quarter Horses and reported asymmetrical ovulation favoring the left ovary (122 ovulations) over the right ovary (79 ovulations) in a population of maiden mares, hypothesizing that this was due to differences in vascularization and greater blood flow to the left ovary, with the asymmetry reportedly eliminated during or after the first pregnancy. However, Rezagholizadeh et al. (2015) documented no significant effect of parity on ovulation incidence between the left and right ovaries, with nulliparous mares showing 48.2% left and 51.8% right ovulations, and parous mares

showing 52.9% left and 47.1% right ovulations (p>0.05). The present study corroborates these findings, showing no significant difference in the ovulation side with respect to parity. The discrepancy with the results of Ginther (1983) may be attributed to breed differences and the methodology used to determine ovulation, as advancements in diagnostic techniques, particularly ultrasonographic evaluation over rectal palpation, have allowed for a more accurate assessment of ovulation patterns in recent studies.

CONCLUSION

In conclusion, this study provides valuable insights into the effect of ovarian laterality on reproductive parameters in Arabian mares, highlighting that the side of ovulation does not significantly impact pregnancy outcomes or foal sex, although age-related declines in pregnancy rates were observed. Further research should integrate genetic analyses to provide deeper insights into the mechanisms underlying the observed patterns of ovulation and pregnancy outcomes.

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