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Research Article

Ovarian follicular dynamics and uterine changes during the ovulatory wave predicts imminent ovulation in Mares

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Abstract

Equine reproduction is unique by having long behavioral estrus and variations in ovarial follicular dynamics that made difficulty to standardize breeding time in mares. There are limited data on equine reproduction and breeding in Ethiopia. An experimental study was conducted at Balderas sport horses and recreational center, Addis Ababa, Ethiopia to determine ovarian follicular dynamics in local and exotic cross breed mares. A daily transrectal ultrasonography was carried out to evaluate uterine changes and ovarian structures for 2 consecutive estrus cycles, as well as teasing scores estimated during estrus. The mean (±SEM) of cross sectional uterine diameter were 44.4±0.5mm and 45±0.5mm for local and cross breed mares respectively; whereas scores of endometrial fold was 3.1±0.1 in local and 2.8±0.1 in cross breed mares with significant difference at P<0.05. The mean (±SEM) diameter of preovulatory follicle for local and cross breed mares were 49.1±1.0mm and 50.1±0.8mm, respectively. Length of estrus was 7.0±0.9 days for local and 6.1±0.6 days for cross breed mares. Fast growth of dominant follicles in cross breed shorten interovulatory interval than local breed mares.A positive correlation of teasing scores, uterine diameter and endometrial fold with diameter of preovulatory follicles (r=0.5) in the present study has been used to estimate imminent ovulation.Ovulation occurred within 6-7days of mean scores of 2.8-3.1endometrial fold, 44.4-45mm of uterine diameter and 2-4 teasing scores for both mares.In conclusion, imminent ovulation can be predicted by measuring uterine diameter, scoring endometrial fold and considering teasing scores in relation to diameter of preovulatory follicles when breeding mares.

Introduction

Reproductive activity of equines in the northern and southern hemisphere is seasonally dependent, in which mares are polyestrous, seasonally long-day breeder [1,2]. In tropical areas seasonal fluctuation causes an increase or decrease in body condition of animals as a result of variation in feed resources, and ovarian activity was also closely following this seasonal pattern [3]. This confirms reproductive behavior in tropical area are affected by forage availability than length of light per day [3-5].

The pattern of ovarian follicular dynamics during estrous cycle in mares vary between breeds under different environmental condition [6-10]. The maximum diameter of the preovulatory follicle at 42.6±1.24 mm in Caspian mares [7], 39.95±4.84 mm in Thoroughbred mares of the United Kingdom [8], 38.8±0.6 mmin mixed breeds of Pony mares [11], showed variable reports and is still difficult to identify the precise time of ovulation and breeding, as every mare do not ovulates with strictest expectations of time.

The overt signs of estrus are attributable to the estrogen production by the follicle on the ovary. As a mare exhibits estrus, estrogen causes a swelling and increased folding in the endometrium [12]. Uterine diameter and endometrial folds become more prominent with increasing diameter of developing dominant follicles as ovulation time approach [13]. This has been used to estimate time of imminent ovulation.

The long periods of behavioural estrus (5-7 days on average), variations in uterine and ovarial follicular dynamics and differences in time of breeding between breeds and individual mares, could confirms the need for study of ovarain follicular dynamics in specific breeds to determine relative time of

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ovulation for reproductive efficiencies.There was very limited data on ovarian follicular dynamics and reproduction as a whole in Ethiopia. Therefore, in this document it is hypothesized that ovarian follicular dynamics with uterine changes can be used to predict imminent ovulation. Therefore, the objectives of this study was to determine ovarian follicular dynamics in local and exotic cross breeds of mares and evaluate its correlation with diameter of the dominant follicles, changes in the uterine and estrus behavior to predict imminent ovulation.

Materials and methods

Study area

The study was carried out in Addis Ababa at Balderas sport horses and recreation directorate of Palace administration, central Ethiopia. The area located at 9°N latitude and 38°E longitude with 2400 meters above sea level. Addis Ababa has an annual rainfall of 1800 ml which falls during the long rainy season extending from June to September and short rain fail extending from March to May. The mean annual maximum and minimum temperature ranges are 23°C and 10.7°C respectively.

Study animals

The study animals were local breed of Selale type mares as characterized by Kefena, et al. [14] and exotic cross bred (Anglo X Arabian) mares which are kept under sport horses and recreational directorate of the palace administration, Ethiopia. A total of 23 apparently health mares (11 local and 12 crossbred) were included in the study. All mares were clinically and ultrasonically evaluated for reproductive soundness before they were included in the experiment. All the mares were properly housed, provided with hay and concentrate. Water was provided ad libitum. They were also allowed to exercise daily for at least an hour.

Ultrasonography

Daily transrectalultrasonographic examinations of reproductive organs were performed for two consecutive estrus cycles as described inGinther[15], using Aloka B-mode, real time scanner with a 5 MHz linear array transducer (Aloka 550, Japan). Uterine cross sectional diameter, number of follicle in each ovary and the diameterof the first three largest follicles were measured using the internal electronic caliper. Ovulation was determined by the disappearance of a largest follicle observed during a previous examination and its replacement by a corpus haemorrhagicum or CL. Ultrasonic endometrial echotexture was scored on a scale of 0-4 based on the degree of endometrial folding (0 for invisible folding; 4as maximal).Teasing scores were estimated on scale of 0-5 and recorded when animals were in estrus, to correlate with size of developing dominant follicle.

Growth rate of dominant follicle was determined by dividing the difference of maximum and minimum diameter of the dominant follicle for duration of growth as defined by Gastal, et al. [16].The non-identity or mathematical method previously described for mares [17] was applied to analyse the remaining ultrasonic follicular data.

Data management and analysis

All collected data were managed in Microsoft excel sheet. Using STATA version 12 (1985-2011 StataCorp LP, College Station, Texas 77845 USA),all data were described using a descriptive statistic; whereas independent sample t-test andone-way ANOVAwere used for analysis of follicular data, uterine diameter, IOI, phases of estrus cycle to evaluate breed differences.

Pearson's correlation was used to evaluate the presence of correlations between uterine diameter, teasing score and development of endometrial fold with size of the dominant follicle as well as growth rate of dominant follicles with IOI. Pearson's correlation coefficient (r) was used to see strength of relationship between the measured parameters by takingextreme values, either +1 or -1.For all data analysis the significance value was set at P<0.05.

Results

The mean (±SEM) of cross sectional uterine diameter were 44.4±0.5 mm for local and 45±0.5 mm for cross breeds; whereas the scores of endometrial fold indicated significant difference at P<0.05. Significantly (P<0.05) higher mean (±SEM) number of follicles were recorded in cross breed (17.4±0.8) mares than the local breeds (13.2±0.4) Table 1.

In the two consecutive estrus cycles of the present study, double ovulations were recorded only in one animal. The mean (±SEM) diameter of preovulatory follicle was 49.1±1.0mm in local and 50.1±0.8mm for cross breed mares. IOI was longer for local (24.3±0.4 days) compared to cross breed mares (22.7±1.0 days). The length of estrus was 7.0+0.9 and 6.1±0.6 days for local and cross breed mares respectively (Table 2).

Table 1: Results of ultrasonic evaluation of the reproductive organs between breeds of mares.

Ultrasonic measurement	Local breed			Cross breed		
	N	Mean +SEM	Range	N	Mean +SEM	Range
Uterus						
Uterine diameter (mm)	152	44.4±0.5	20.2-56	156	45.0±0.5	27.1-58.4
Endometrial fold score[a]	141	3.1±0.1	0-4	136	2.8±0.1	0-4
Right Ovary (ROV)						
Number of follicles[a]	94	6.9±0.3	3-16	72	8.7±0.6	2-17
1st largest Follicles(mm)	184	28.2±0.8	5.2-54.4	163	28.9±0.9	10.9-57
2nd largest Follicles(mm)[a]	182	17.8±0.5	4.2-38.1	161	19.4±0.5	6.4-35.7
3rd largest Follicles(mm)[a]	180	13.7±0.4	3.2-29.3	160	15.3±0.4	5.5-30
Left Ovary (LOV)						
Number of follicles[a]	94	6.3±0.2	3-14	72	8.6±0.4	3-16
1st largest Follicles(mm)	188	29.4±0.9	8.7-58.1	165	28.6±0.8	9.2-55.7
2nd largest Follicles(mm)[a]	181	18.5±0.5	5.7-33.4	162	21±0.6	5.4-49.1
3rd largest Follicles(mm)[a]	178	14.3±0.4	4.1-28.2	162	16.4±0.5	4.9-36.1
Total number of follicles[a]	94	13.2±0.4	6-24	72	17.4±0.8	3-30

N: Number of measurements; SEM: Standard error of the mean; Superscript a represent P<0.05 between breeds

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Changes on uterine and ovarian follicles were characterized during late luteal and estrus phases of the cycles. A cross sectional uterine diameter, endometrial fold score and number of follicles showed significantly higher measurements in the second cycle than first estrus cycle. similarly, the diameter of preovulatory follicles were higher in the second cycle (50.9±0.9mm) than first cycle (48.1±0.9mm) with statistically significant difference at P<0.05 (Table 3).

A positive correlation was demonstrated between diameter of largest follicles and uterine diameter (r=0.5), diameter of largest follicles and endometrial fold (r=0.56), and diameter of largest follicles and teasing score (r=0.69).Correlation between diameter of largest follicles and IOI (r=0.19)and between the growth rate of dominant follicles and IOI (r= -0.17), were not strong (Figure 2).

Ovulation occurred within 7.0±0.9 days for local and 6.1±-7 days for cross breed with mean scores of 3.1±0.1 and 2.8±0.1 endometrial fold for local and cross breed mares respectively. In addition uterine diameter of 44.4±0.5mm for local and 45±0.5mm for cross breed and 2-4 teasing scores for both breeds of mares were evaluated at occurrence of ovulation. The mean endometrial fold and teasing scores had positive correlation with increasing uterine diameter.

Discussion

In this study the mean (±SEM) of cross sectional uterine diameter between local and cross breed mareshave no significant difference. Previous studies of Lemma, et al. [18] and Griffin and Ginther [19] reported mean uterine diameter of lower size than present study. In study of Griffin and Ginther [19], uterine dimeter was increased significantly between 11 and 21 days of estrus cycle. This implies larger diameter at end of the cycle was indicator of relative time of ovulation in mares. The difference in diameter will be affected by season of the study, difference in breed or technical error of measurement. Higher uterine diameter in the present study will be due to delayed time of ovulation after the follicle size reach the standard (> 35mm) which will cause continues growth of uterine and ovarian structures.

A significant mean differences of endometrial fold scores were found between breeds at P<0.05.Samper [20] found that smaller average value of endometrial folding immediately before ovulation; indicates approaching time of ovulation. Even level of the scores different between breeds, the present result was agreed with the scientific fact of endometrial folding in mares' correlates with estrogen production in the dominant follicle and usually starts to be noticeable on day seven before ovulation; then maximum near to time of ovulation and subsequently diminishes and disappears after ovulation [12].

In each ovary the mean diameter of 1st largest follicle compared between breeds and resulted in closely similar diameter. The mean (±SEM) on both ovaries ranges from 28.2±0.8mm to 29.4±0.9mm with non-significant differences between breeds. The mean diameter of 2nd and 3rd largest follicle showed significant difference (P<0.05) between breeds with higher diameter in cross breed in respective to each ovary. The variation will be due to difference in level of plasma circulating FSH and other inhibitory factors between breeds that affect size of subordinate follicles [21].

The number of counted follicles in each side of ovary had a significant difference (P<0.05) between breeds with frequently higher number counted in cross breed than local breed mares. There is also slightly higher number of follicles on right ovary than left ovary. The overall mean (±SEM) in number of follicles were 17.4±0.8 in cross breed and 13.2±0.4 in local breed mares. Similar result was reported by Lemma, et al. (2015) for cross breeds mares with 16.2±6. ltotal number of follicles. Higher number of follicles in cross breeds of mares might be one indicator of the differences in fertility level between breeds.

Diameter of preovulatory follicle in local and cross breed mares of the present study indicated mean (±SEM) of 49.1±1.0mm and 50.1±0.8mm respectively. This is comparable with the research work of Dolezel et al. [22], who reported the diameter of 48mm in Czech warmblood mares. Other researchers like Dimmick, et al. [23], Ginther, et al. [24], Ginther, et al. [25] and Camargo, et al. [9], reported lower preovulatory follicle diameter of 40-45mm than the present study on the day before ovulation. The difference in diameter of preovulatory follicles might be affected by breed, season of the study [2], number of ovulation as single or double ovulation per cycle [25], as well as age of animals as confirmed by Davies Morel, et al. [8].

Table 2: Growth characteristics of preovulatory follicle and length ofinterovulatory intervalin local and cross breed mares.

Measurement	Local breed			Cross breed		
	N	Mean + SEM	Range	N	Mean + SEM	Range
Diameter of Pre-ovulatory follicle (mm)	22	49.1±1.0	38-55.6	22	50.1±0.8	42.7-57
Growth rate of dominant follicle (mm/day)	21	2.8±0.1	1.4-3.8	18	2.9±0.2	1.2-4.1
IOI (days)	9	24.3±0.4	22-26	10	22.7±1.0	18-27
Length of estrus (days)	9	7.0±0.9	3-13	10	6.1±0.6	4-10
Length diestrus (days)	9	17.1±1.0	12-20	10	16.9±0.6	14-22

N: Number of measurement; SEM: Standard errorof the mean

Table 3: Mean uterine diameter and ovarian follicles between consecutive estrus cycle.

Ultrasonic measurement	First cycle			Second cycle		
	N	Mean + SEM	Range	N	Mean + SEM	Range
Uterine diameter (mm)[a]	106	43.6±0.6	20.2-56	65	48.6±0.4	33.9-54.6
Endometrial fold score[a]	97	2.9±0.1	0-4	65	3.4+0.1	2-4
Diameter of Preovulatory follicles(mm)[a]	21	48.1±0.9	42.7-58.1	20	50.9±0.9	38-55
Diameter of 1st largest follicles on ROV(mm)	138	32.4±0.8	20-57	99	34.3±1.1	20.4-55.1
Diameter of 1st largest follicles on LOV (mm)	134	34.9±0.9	21.2-58.1	93	33.8±1.0	21.2-55
Number of follicles on ROV	45	7±0.6	2-16	62	7.7±0.5	3-16
Number of follicles on LOV[a]	42	5.7±0.4	3-12	62	7.6±0.5	4-16
Total number of Follicles[a]	44	13.1±0.9	6-27	62	15.5±0.7	6-27

N: number of measurements, SEM: Standard error of the mean; Superscript a represent P<0.05 for mean difference between estrus cycle

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Growth rate of dominant follicles were slightly higher in cross breed than local breed mares; this resulted in higher IOI in local than cross breed mares. Relatively similar growth rates of dominant follicles were reported by experimental work of Gastal, et al. [11], Aurich [26] and Dolezel, et al. [22]. Rate of follicle growth can be influenced by stages of estrus cycle at which the diameter of follicles measured, as well as induction of ovulation and estrus in addition to breed differences.

The mean length of IOI in present study was closely similar with the findings of Griffin and Ginther [19] and Shirazi, et al. [7] who reported an interval of 23 and 22.1±0.43 days respectively. A minor difference between the studies will be due to the effect of breed, age of study animals as growth rate of the dominant follicle lower and IOI may be longer in old mares than young and middle-aged mares [27]. Induction of ovulation decreasesthe size of the preovulatory follicles in comparisonwith natural ovulation and this shortens again the IOI [28].

The length of estrus indicated close similarity between both breeds with slightly longer estrus and diestrus phases in local than cross breed mares. The present result was consistent with the report of 5-7 days of estrus with cycle length of 22 days [26], but higher than diestrus period of 14 to 15 days [29,30]. The differences might be due to functional differences of ovary between breed of mares. In addition to this, season affects the duration of follicular phase in which the duration of estrus decreases during the summer, which most likely represents an acceleration of folliculogenesis before ovulation [6].

Ovarian follicles and uterine structures were characterized between two consecutive estrus cycles in the present study. Accordingly, a cross sectional uterine diameter in the second cycle were higher than the first cycle. Similarly, endometrial fold score in second cycle were significantly higher than that of the first cycle at $P<0.05$. The diameters of preovulatory follicles were higher in the second cycle than first cycle with statistically significant difference. The total number of follicles in both ovaries resulted in mean (±SEM) of 15.4±0.4 for second cycle and 13.1±0.9 for first cycle with significant difference. The variation in activities of ovarian function from one cycle to another in the present findings,related to justification in the study of Lemma, et al. [3], who stated follicle size and number would beaffected by feed availability and body conditions of the study animals from one season to another.

Pearson's correlationused to correlate different parameters during estrus cycle of the mares to see their relationships with developing dominant follicles. The study revealed a positive correlation of cross sectional uterine diameter and endometrial fold scores with diameter of developing dominant follicles. A similar finding was reported by Lemma, et al. [13], who found strong positive correlation of indicated parameters in tropical jennies (Equusasinus). This study also proves the previous description of, follicle size has been a good parameter to determine the timing of breeding in horses [31] and endometrial fold scores were suggested as a reliable predictor of imminent ovulation [22].

Teasing scores showed strong correlation with diameter of developing dominant follicles (Figure 1; r=0.69).Mares have different level of receptivity to stallion through estrus period for which most managers' use teasing in estrus detection for breeding determination or AI in addition to rectal palpation and ultrasonography [32]. As maximum diameter of preovulatory follicles are an indicative of a relative time of ovulation [31]; a strong correlation with teasing scores in the present study confirms, implementation of teasing in combination with rectal palpation and ultrasonography is best method to determine breeding time and cost effective if used alone with good experience.

Growth rate of dominant follicles have inverse relationship with IOI (i.e. this implies faster growth of the dominant follicle shorten IOI and the vice versa). However, growth rate and days of IOI can be affected by age [27], stage of estrus cycle and induction of ovulation [28].

Conclusion

A closely similar preovulatory follicles and cross sectional uterine diameter were measured between the two breeds of mares; but cross breed had significantly higher number of follicles than local breed mares.A positive correlation of cross sectional uterine diameter, endometrial fold score and teasing scores with diameter of preovulatory follicles can have used to estimate the time of ovulation from these findings and hence can fix the best time for AI. Therefore, correlation of behavioral estrus/teasing with diameter of preovulatory follicle should be considered to determine imminent ovulation in combination with uterine diameter and endometrial fold.

Ethical approval

Ethical clearance for this study was obtained from animal research ethical review committee of Addis Ababa University, College of Veterinary Medicine and Agriculture with Ref. No CVM/ERC/15/05/10/2018.

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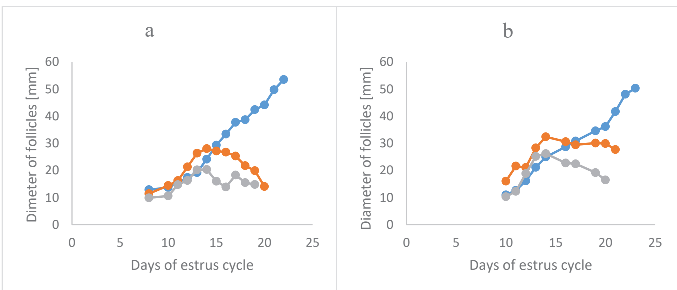

Figure 1: Ovarian follicular activities in local and cross breed mares during ovulatory wave: a) Mean diameter of first three largest follicles in cross breed mares b) Mean diameter of first three largest follicles in local breed mares. Both of the figures showed how subordinate follicles become regressed and the largest follicles become dominant for further growth to be ovulated.

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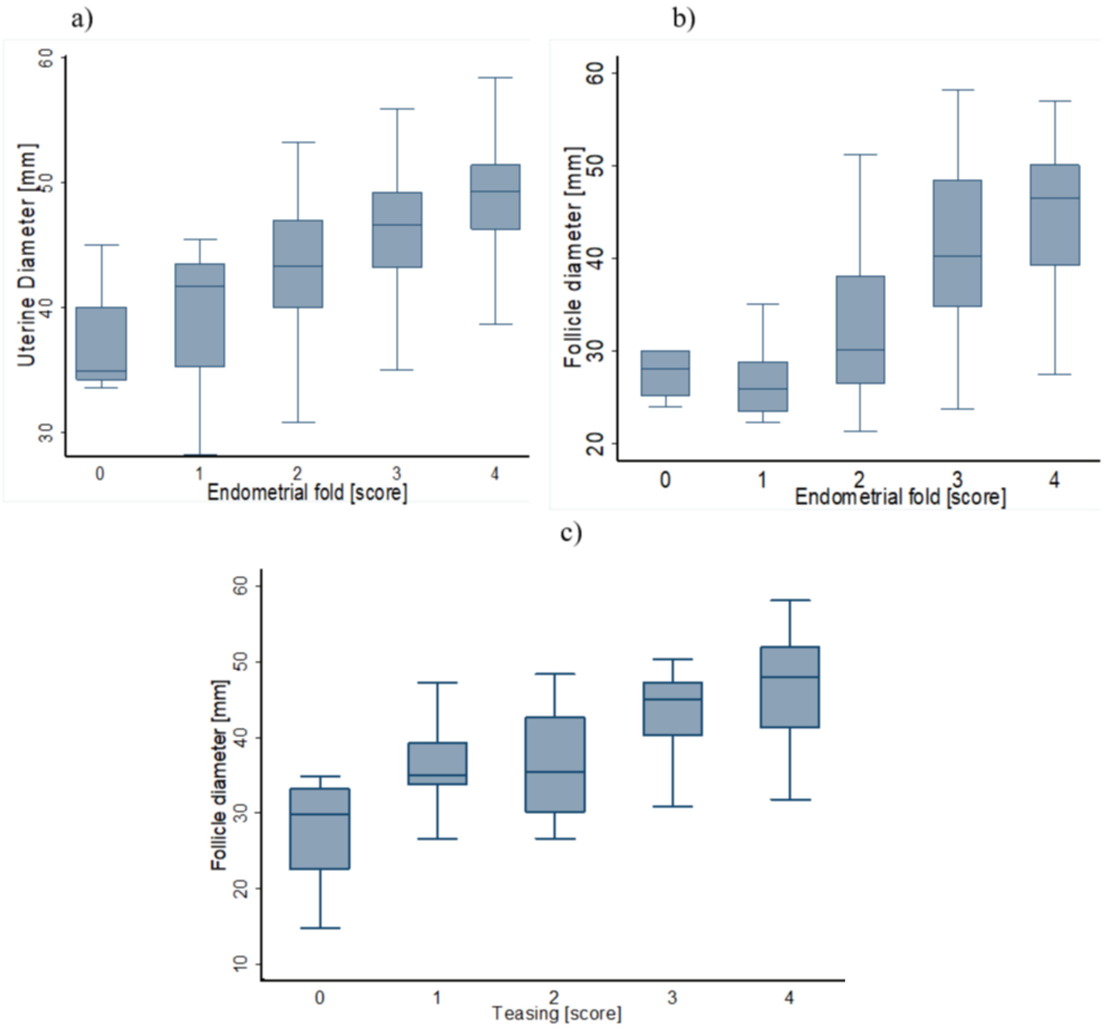

Figure 2: Correlation between uterine parameters and diameter of the dominant follicle: a) endometrial fold and uterine diameter b) Endometrial fold and follicular diameter c) Follicular diameter and teasing score.

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