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Research Article

Ovarian Follicular Dynamics during the Estrous Cycle in Jennies in Upper Egypt

R. I. Derar and H. A. Hussein

Department of Theriogenology, Faculty of Veterinary Medicine, Assiut University, 71526 Assiut, Egypt

Correspondence should be addressed to R. I. Derar, derar40@yahoo.com

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The objective of the current study was to describe follicular dynamics in Egyptian Jennies throughout the estrous cycle. In this experiment, 8 estrus cycles in 8 cyclic Jennies were studied from February to June using ultrasonography. The result revealed that one follicular wave per cycle was recorded throughout the studied period. Dominant follicle (DF) was firstly detected at -0.80 ± 0.84 day in Jennies. The growth rate of DF was 2.32 ± 0.18 mm/day. Left ovulations were nonsignificantly ($P = .07$) more than right ovulations (55.6% versus 44.6%). The CL was firstly detected at D 2.58 ± 1.2, developed in a rate of 1.19 ± 0.07 mm/day, reached a maximum diameter of 30.77 ± 1.28 mm at D 13.0 ± 0.70, and started to regress on D 17.05 ± 0.64 with a mean regression rate of 1.75 ± 0.17 mm d^{-1}. Results of the present study indicated that Jennies had one follicular wave per cycle. The Day of the cycle has a significant effect on the number of different classes of the ovarian follicles, but not large ones. Ultrasonographic characteristics of the preovulatory follicles could be useful to predict ovulation. CL developed and regressed in a slow rate.

1. Introduction

Donkeys (*Equus asinus*) are enjoying increased popularity as working livestock in Egypt. The farmers still depend on this animal in carrying weights, drag small cars, and other field works. In comparison to the mare, few studies have been reported regarding the estrous cycle of the jennet. The reproductive parameters such as follicular wave patterns [1], multiple ovulations, the size of ovulatory follicle, ovarian responses to exogenous gonadotropin stimulation [2, 3], the incidence of diestrus ovulation [4], the pattern of antral formation [5], and some other physiologic characteristics related to reproductive system in mare were studied [6–9]. The follicular wave patterns, size of ovulatory follicle and ovulations still have to be determined in Jennies [10]. However, little is known about the differences between Egyptian and other Jennies as well as mares in these respects—a lack of basic knowledge that hinders the use of reproductive technologies. Understanding the pattern of ovarian follicular development is seen as an important step leading to the development of techniques that maximize fertility in Jennies. To improve reproductive efficiency in Jennies, further basic information about the estrous cycle as well as follicular dynamics is needed. Therefore, in order to increase our knowledge about reproductive physiology in this species, this study was conducted to describe and characterize the patterns of ovarian antral follicle turnover and corpus luteum growth and regression throughout the estrous cycle in Jennies.

2. Materials and Methods

2.1. Animals and Management

2.1.1. Jennies Data. Eight clinically healthy, nonlactating native breed Jennies, aging 5–10 years, weighing 160–195 kg were used in this study. The animals were cyclic and showed signs of estrus regularly. The study was carried out on the same group of animals over one complete estrus cycle for each Jennies from February to June. Daily observations for detection of estrus in jennets (mouth clapping, urination, vulvar activity, and accepting male) were conducted. Teasing was carried out to detect estrus using Jack ass of high libido.

2.1.2. Feeding. The animals were fed Egyptian clover (*Trifolium alexandriunm*) from December till May and had free access to water and mineralized salt. The animals were provided with concentrated ration and wheat straw ad libitum.

2.1.3. Management. Animals were kept under natural light in an open shelter and outdoor paddock in Animals Teaching Clinic, Faculty of veterinary medicine, Assiut university, Assiut, Egypt (latitude: 2710 58 N; longitude 3310 58 E; altitude 37 m) under the prevalent environmental conditions.

2.2. Ultrasonic Examinations

2.2.1. Ultrasound Equipment. Ovaries were monitored transrectally once a day with an ultrasound scanner equipped with a 6/8 MHz changeable linear-array transducer (Pie Medical, 100 LC, Maastricht, Netherlands).

2.2.2. Start of Monitoring. Ultrasonic examination was carried throughout 8 interovulatory intervals. At each examination, the number, diameter, and relative position of all follicles 6 mm in diameter and corpora lutea (CL) were recorded and sketched on ovarian charts to analyze the pattern of growth and regression. When a follicle or CL was not spherical, a mean diameter of two diameters was taken, then the averages were calculated. Follicles were ranked into three size classes: small follicle (6–10 mm diameter), medium-sized follicles (10–25 mm diameter), and large follicles with diameter of 25 mm.

2.2.3. Follicular Data Analysis. The follicular wave was monitored daily, and all follicles were tracked to maintain individual identity. The relative locations of follicles and corpus luteum were used as references in identifying and tracking individual follicles. Day of emergence of a follicle was defined as the day before the follicle first exceeds 6 mm [11]. The duration of growth of a follicle was the time taken by that follicle to grow from 6 mm in diameter to its maximum diameter (MD). The growth rate (GR) was considered as the difference between maximum and minimum diameter of the largest follicle divided by the duration of its growth. The duration of follicular atresia was the time taken by that follicle to regress from maximum diameter to <6 mm in diameter. The atretic rate (AR) was taken as the difference between maximum and minimum diameter of the DF divided by the time taken for atresia. The dominant follicle (one that grows to a preovulatory size of 25 mm) and the largest subordinate follicle (one that grows to a moderate size and is regressed) were chosen retrospectively according to the maximum attained diameters. The beginning of diameter deviation (divergence) between the future dominant and largest subordinate follicle was defined as the day the 2 follicles began to differ in growth rates [11, 12]. Thus, the beginning of deviation refers to the examination preceding the first change in differences in diameters between the 2 follicles. Days of ovulation were regarded as the days on which a large antral follicle, 25 mm diameter, that had been identified and followed for several days was no longer observed. Follicles were categorized [13] into small (6–10 mm), medium (11–25 mm), and large (>25 mm). The following characteristics of follicular waves, for each she-donkey, were determined: (1) mean number of follicular waves; (2) mean number of small- and medium-sized follicles; (3) mean diameter of small-, medium-, and large-sized follicles; (4) mean maximum diameter attained by the DF of the wave; (5) mean growth and regression rates of the DF of the wave.

2.2.4. Corpus Luteum. The corpora lutea were examined and an image of the largest cross-sectional area was frozen and estimated. The first day of detection, growth rate, the day of maximum diameter, the first day of regression, and the regression rate were recorded.

2.2.5. Statistical Analysis. The durations of the estrous cycle, estrus, and diestrus were represented as means. Data were normalized to days of ovulation for analysis and presentation. Follicular and luteal data were also analyzed for period effects using repeated measure analysis of variance (ANOVA), with Fisher's protected least significant difference (LSD) as the post-ANOVA test [14]. Chi square was used to determine whether the largest follicle and the corpus luteum occurred more frequently on the same or the opposite ovary. Relationships between location of a largest follicle and location of the next one were examined by Chi-square-goodness-of-fit and frequency distribution analysis. Data were expressed in mean SEM, and the significance was set at $P < .05$.

3. Results

The first day in which jenny showed estrous signs was considered day 1. The length of the IOI, estrus, and the diestrus periods were 24.25 1.26 (range 22–28), 8.6 0.61 (range 4–11), and 17.25 0.72 days (range 14–21), respectively. A single follicular wave was recorded in all the studied IOIs. The length of the follicular wave was 25.3 1.63 day. The mean numbers of small- and medium-sized follicles (Figure 1) varied significantly among days of the estrous cycle ($P = .01$).

The day of follicular deviation of the DF was recorded on D 0.80 ± 0.84 with growth rate of DF was 2.32 ± 0.18 mm d^{-1} and maximum diameter of 36.13 5.72 mm at D 17.60 11.14 (Figure 2). The preovulatory follicle underwent a pronounced change in shape from approximately spherical to a mainly conical in 66.6% (6/9) of instances in Jennies (Figure 3). The number of the largest follicles located in the left versus the right ovary did not differ significantly among wave categories. The frequency in which the largest follicles were in the same (31.8%) versus the opposite (68.2%) ovary to the location of the CL differed significantly ($P = .01$). The largest follicles were located in the same or opposite ovary to the previous largest follicle in equal frequencies (not significantly different from equality). Four out of nine (44.4%) ovulations were from the right ovary and 5/9 (55.6%) from the left ovary. Out of all the recorded cycles, only one double ovulation was noticed. The CL

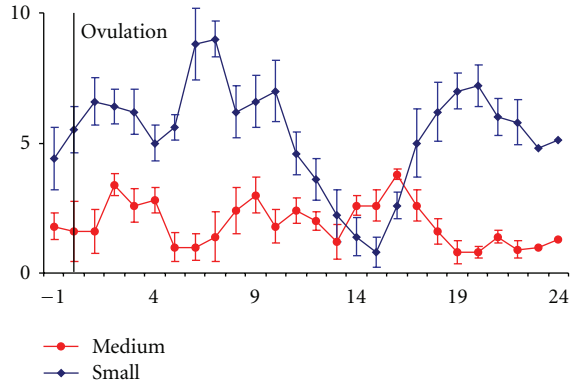

Figure 1: Number of medium-sized follicles during the IOI in Jennies.

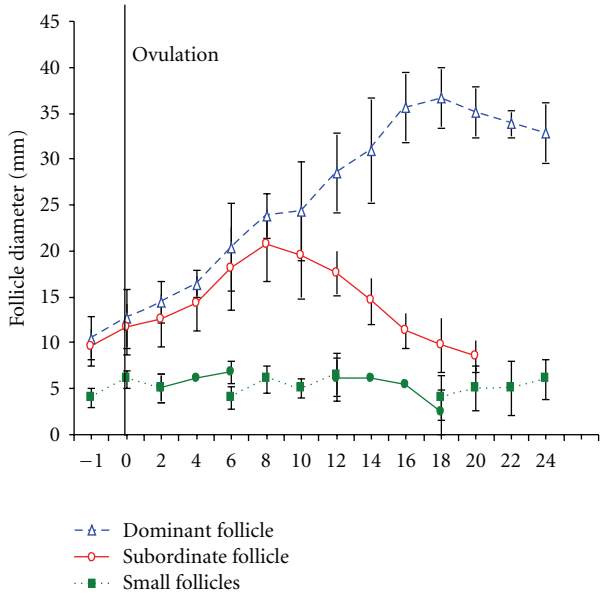

Figure 2: Follicular wave in Jennies during the studied IOI, one FW.

was firstly detected at D 2.58 1.2, developed in a rate of 1.19 0.07 mm d^{-1}, reached a maximum diameter of 30.77 ± 1.28 mm at D 13.0 0.70, and started to regress on D 17.05 0.64 with a mean regression rate of 1.75 0.17 mm d^{-1} (Figure 4).

4. Discussion

In the current study, the mean IOI in native Jennies was 24.25 1.26 with longer diestrus period (17.25 0.72 d). The obtained results reported here were in general agreement with that observed in other studies for mammoth Jennies [6], Catalonian Jennies [15], and other breeds [7, 16–18]. There is general agreement that, while the duration of estrus is similar between Jennies and mares, the duration of diestrus is longer in Jennies [7–9, 17, 19, 20]. The estrous cycle of Jennies is less affected by season than that of either ponies or horses, with a high percentage of Jennies cycling throughout the year [8]. Ultrasonic observations in the present study would support the theory of dominance in Jennies. Throughout the studied IOIs, one follicular wave per cycle was recorded. The length of the follicular wave was 25.3 1.63 day [21]. Two (minor anovulatory and major ovulatory) waves were identified per estrous cycle in pony mares [22] and mares [23–25]. In horses, the follicles of the ovulatory wave undergo several days of a common-growth phase that ends at the beginning of follicle deviation, wherein a dominant follicle continues to grow and other follicles begin to regress [11]. The day of emergence of the future ovulatory follicle is obscured in mares during the common-growth phase in about 25% of ovulatory waves from overlapping by follicles of a previous wave [23]. Diameter of the largest follicle and number of large (>20 mm) follicles began to increase significantly 7 days prior to ovulation, with maximal diameter in the day before ovulation [7], these results were similar to those reported in the present study. The recorded results regarding the growth rate of preovulatory follicle to reach a maximum diameter was compatible with other reports [6, 18]. The day of follicular deviation of the dominant follicle (DF), its growth rate, maximum diameter, day of maximum diameter deviation day were similar to findings of previous studies for native Egyptian Jennies [26], but greater values were reported for mammoth (41.3 ± 1.3 mm [6] Catalonian [15], standard [18] and Martina Franca [20] Jennies). Estradiol is a candidate for involvement in the mechanism that causes follicle deviation in mares [27]. Deviation begins when the future dominant and largest subordinate follicles are approximately 22.5 and 19.0 mm, respectively [24, 25]. Number of small- and medium-sized follicles during the earlier periods (emergence) of the follicular wave in Jennies and their later reduction during the deviation of the dominant follicle support the concept of negative effect exerted by the largest follicle of the wave on the rest of the follicles. It was cited that diameter of the largest follicle and number of large (>20 mm) follicles began to increase significantly 7 days prior to ovulation on expense of the small- and medium-sized follicles, with maximal diameter (averaging 36 mm; range 30 to 40 mm) achieved in dominant follicles the day before ovulation [18]. The follicular growth rates in Jennies [10] in estrus averaged 2.7 mm per day, with a conspicuous decrease in other follicle classes (small and medium). Ovulation usually occurred when follicles reached 41 mm diameter. The number of follicles in the smallest diameter classification (2–5 mm) was significantly and positively correlated within individuals [27]; the extent of repeatability diminished as the small follicles entered the larger classes in the dynamics of the turnover involved in follicle growth and atresia. During the common growth phase between emergence (6 mm largest follicle) and deviation (mean 22.8 ± 0.5 mm largest follicle) in the ovulatory wave, the numbers of follicles 6 to 9 mm and 10 to 14 mm were measurably reduced.

The size of preovulatory follicle at the onset of estrus and its growth rate could influence the length of estrus before ovulation. The growth rate of DF in Jennies was 2.32 ± 0.18 mm d^{-1} in the present study. In miniature ponies, the growth rate from emergence (mean day 12) of the future ovulatory follicle at 10 mm to maximum diameter (3.0 ± 0.3 mm/day) was greater than the rate from maximum

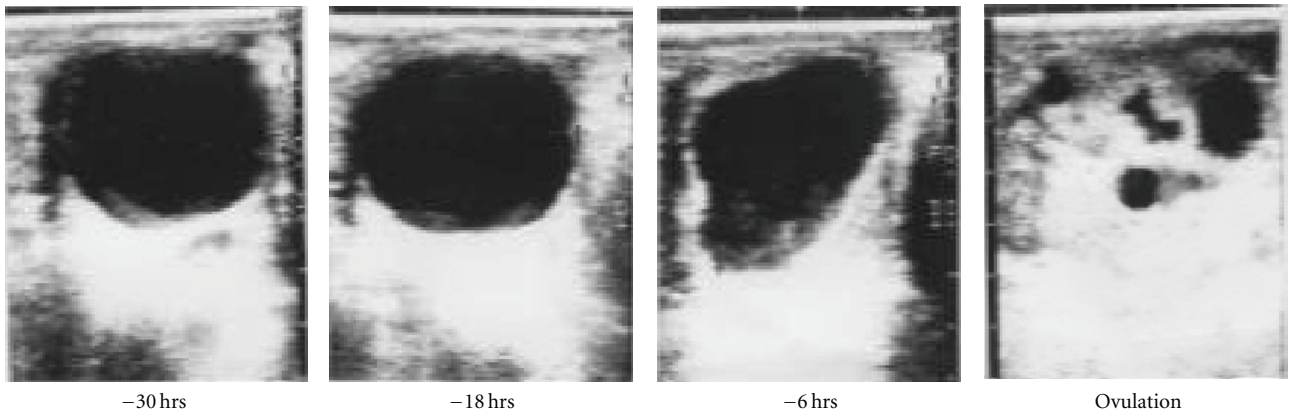

Figure 3: Ultrasonographic appearance of the preovulatory follicle (morphodynamic changes) during the estrus phase, shortly before ovulation in she-donkeys.

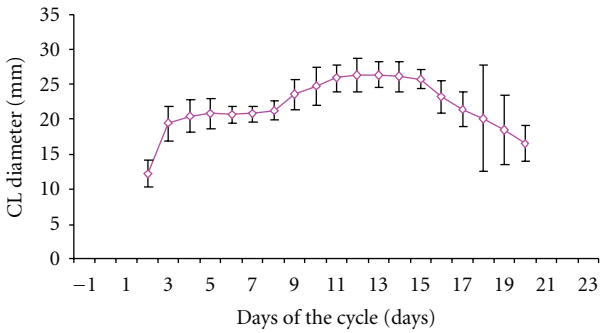

Figure 4: Changes in cyclic CL diameter in Jennies during the estrus cycle.

diameter [22] to ovulation (−1.2 ± 0.8 mm/day). In equidae, the larger the follicular diameter on the first day of estrus is', the sooner the follicle will ovulate and the shorter the estrus period will be [28]. The growth rate of the ovulatory follicle between days −6 and −3 was greater than between days −3 and −1 for Jennies. The reduction in growth rate of the ovulatory follicle between maximum diameter (1 or 2 days before ovulation) and ovulation in Miniature ponies is consistent with what has been reported for Catalonian Jennies [15], larger ponies [29] and horses [24, 25, 27, 30, 31]. It was mentioned that the maximum diameter was attained on day −2 (46%) or day −1 (54%), reflecting the mean diameter decrease before ovulation [27]. The preovulatory follicle in the present study required is between 5 and 3 days to prepare for ovulation. This longer time is quite different from the time needed in mares or other breeds of Jennies. Breed and time of the year when the study conducted was may be detrimental factors. The mean diameter at ovulation was 42.9 mm in Martina Franca Jennies [20], greater than the mean preovulatory diameter seen in the present native Jennies. However, in other donkey breeds, the mean diameter of preovulatory follicles is reported to be smaller at around 36–41 mm [7, 10, 17]. These differences may be associated with the smaller size of these donkeys.

The pronounced changes in shape of preovulatory follicles, from spherical to nonspherical, sometimes during the preovulatory follicle, were due to a decrease in fluid pressure within the antrum [15, 28, 32–34]. Occurrence of irregularities in the follicular outlines was associated with a decreased pressure within the preovulatory follicle [15, 34]. Findings observed here and in the previously above-mentioned studies help greatly to identify and predict ovulatory follicles ultrasonically. A gradual thickening of the follicular wall and changing follicular shape from circular to irregular were found as ovulation approached in Jennies [10]. Data on preovulatory changes in estrous behavior, follicle size, follicle texture, the echographic appearance of the follicle and uterus, and uterine tone were subjected to stepwise logistic regression analysis to detect predictors of ovulation [35]. The logit function showed the best predictors to be follicle size, follicular texture, and estrous behavior. Certain combinations of these three variables allow the prediction of ovulation within 24 h with a probability of >75%.

CL in Jennies was firstly detected on D 1.9 0.84, grew in a rate of 1.19 ± 0.07 mm d^{-1} with a maximum diameter of 26.77 ± 1.28 mm recorded at D 13.0 ± 0.70 and started to regress on D 17.05 ± 0.64 with a mean regression rate of 1.75 ± 0.17 mm d^{-1}. The slower rates of growth and regression of the CL in the present study may explain the quite longer diestrus period in native Jennies compared with mares (unpublished data). Regardless of the duration of estrus, the corpus luteum is functional for a constant length of time [36]. Therefore, most of variability in the length of the estrous cycle is due to variability in length of estrus [37]. A prolonged CL is frequent factor that may influence the estrous cycle length. The luteal phase may range from 35 to 90 days instead of normal 14–16 d. In the present paper, most of the CLs observed showed a homogeneous echogenic texture; in contrast, 50–70% of mares have CLs with a nonechogenic central area [38]. In horses [35], CL area decreased at the luteolytic period at a greater rate than during the preluteolytic period. Moreover, the decrease in progesterone was about sixfold greater during the luteolytic period than during the preluteolytic period. The slower

decrease in CL area than in progesterone concentrations during the luteolytic process accounts for the greater percentage of maximum value for CL area than for progesterone after the end of the luteolytic period. 44.4% of ovulations were from the right ovary and 55.6% (5/9) from the left ovary in the present study. The frequency of ovulation from the left ovary was slightly higher than the right ovary in accordance with other breeds (5–20% more ovulations from the left ovary) [1, 15, 26, 34, 39, 40]. However, no significant difference in the number of ovulations involving the left and right ovaries [35] in mares (52.63% compared to 47.37%, resp.; $P > .05$) was observed.

Only one double out of 9 ovulations was recorded for native Jennies in the present study. Data regarding multiple ovulations in native Jennies in Egypt are lacking, but other reports handled this aspect in other foreign breeds. The incidence of multiple ovulations for standard Jennies has been reported to range from 5.3% to 31.8% [18]. The incidence of multiple ovulations in a herd of mammoth Jennies was found to be higher (61%) than that reported for standard Jennies, with several of the multiple-ovulating Jennies doing so on several estrous cycles [18]. The incidence of double ovulations in the horse has been found to be affected by breed, with larger breeds of horses having a higher incidence than smaller breeds of horses or ponies [22]. Multiple ovulations are characterized by significant repeatability within individual mares and suggested that the condition may be heritable [23]. The higher incidence of multiple ovulations reported in the herd of mammoth Jennies may have been a reflection of size of the animals or may have been due to selection of certain family lines. Apparently, the majority of Jennies having multiple ovulations remain in estrus until after the final ovulation [15, 18]. The Catalonian and Mammoth breeds have the highest incidence of double ovulations (61 and 44.3%) recorded for donkeys. This suggests that double ovulations in Jennies are affected by breed [15].

5. Conclusion

In conclusion, estrus cycle in Egyptian Jennies is characterized by longer diestrus phase. Estrus cycle is characterized by single ovulatory follicular wave. Preovulatory follicles developed in slower rate but lasted for a longer time. Although nonsignificant, left ovulations are more frequent than right ones. Day of the cycle has a significant effect on the number of different classes of the ovarian follicles, but large ones. Ultrasonographic characteristics of the preovulatory follicles could be useful to predict ovulation. CL developed and regressed in a slower rate and lysed later. Further studies are needed in Jennies to counteract the lack of basic knowledge that hinders the use of reproductive technologies in this species.

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