Follicular Dynamics in Pubertal Arab Mares during Spontaneous Estrus

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ABSTRACT:

This study aims to study the follicular changes during the estrous phase of 2 years pubertal mares showed previously signs of estrous and corpora lutea were detected on their ovaries. Five fillies of 2 years old were subjected to daily ultrasonographic examination for 18 days. Follicular dynamics were studied during estrus from day 6 before ovulation (day -6) till day of ovulation (day 0). Follicles were classified into small (<10-15mm), medium (>15-<20) and large (>20). Number and diameter of each follicle was measured. Data was subjected to simple one way ANOVA to study the effect of day on number and growth rate of each follicle class. Results revealed that no significant variation was observed in number of all follicle classes. The dominant follicle after deviation to dominance starts to grow from >26mm in diameter to reach a maximum diameter of 42.3 mm on day of ovulation. At least one large follicle is dominant and the other large follicle is subordinate three days before ovulation. Compared to the day 6 before ovulation, the day before ovulation has significantly low large follicle number. There was a decrease of number and diameter of medium follicles on day 2 before ovulation. This study indicated that after ovulation fillies became much similar to mature and older mares. High genetic value fillies could be subjected to oocyte collection programs before enrolling her to either breeding program or performance.

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1. INTRODUCTION

Puberty in fillies is generally considered to be at approximately 12 to 15 months if the animals were born early in the breeding season. Fillies born late may reach sexual maturation during the second spring of life (Ginther, 1992). Age of puberty is affected by season of Thoroughbred. The age at onset of puberty in spring- and autumn-born Thoroughbred foals raised on pasture in southern hemisphere was 291-408 days (9-14 month) and 212-270 days, (7-9 month) respectively. However, the mean date at onset of puberty was not significantly different between spring- and autumn-born horses, with puberty occurring in October of New Zealand (Brown-Douglas et al., 2004). Follicle populations were studied during age 2-10 months in 10 spring-born pony fillies. During 5-12 weeks, the diameters and numbers of follicles increased progressively during age 2-4 months. A plateau in follicle activity occurred during 5-7 months. During 8-10 months, follicles grew to > 10 mm. Waves of follicular growth were identified during a 30 day periods by significant increases in the diameter of the 10 largest follicles. The waves did not partition into dominant and subordinate follicles (Nogueira and Ginther, 2000). Ponies born during the previous summer and early autumn tend to have fewer ovulations and shorter breeding seasons than those born during the spring, whereas those born late in August and September do not ovulate during their first breeding season (Wesson and Ginther, 1982).

Development of antral follicles in horses is characterized by the periodic growth of follicular waves which often involve the selection of a single dominant follicle. If properly stimulated, the dominant follicle will complete development and eventually ovulate. Periodic development of follicular waves continuously occurs during most of post-natal life in the mare and is influenced by factors such as stage of estrous cycle, season, pregnancy, age, breed and individual so that different types of follicular waves (minor or major, ovulatory or anovulatory) and different levels of activity within waves may develop under different physiological conditions (Donadieu and Pedersen, 2008). Significant increases in the diameters of 6 largest follicles, excluding the dominant follicle, were used to detect the emergence
of follicular waves. Emergence of the primary wave occurred later in the oldest pony mares (≥20 years) than in the younger pony mares (5 to 7 and 15 to 19 years). The defect in the old mares, therefore, apparently involved the ovaries which represent a deficient in a follicles number’s (Ginther et al., 1993). In spite that, no effects of age (5 to 18 years) were detected on oocyte morphology, but the interovulatory intervals (IOI) was 1 day longer in the old mares than in the younger mares and was associated with a slower growth rate of the ovulatory follicle. The old mares had diminished follicle activity, as indicated by significantly smaller and fewer follicles. Moreover, old mares were not approaching senescence, as indicated by regular lengths of the interovulatory intervals (IOI; 19-27 days) during the period May-October (Ginther et al., 2008). The advent of ultrasound helped theriogenologists around the world to understand more about ovarian follicular dynamics, ovulation and ovulation failure in equine.

Recently, follicle profile was studied in ovulatory and non-ovulatory pubertal female pony (Nogueira, 2004). No data was available about follicular dynamics in pubertal fillies because native and Arabian mare’s needs delicate handling during such study and younger inexperienced fillies to either rectal or ultrasound examination will need much more experience in both rectal and ultrasound examination, in addition to a suitable operator hand size to reach ovaries. This study aims to study follicular dynamics in fillies to predict their forthcoming breeding and select them for breeding programs.

2. MATERIALS AND METHODS

Animals and ultrasound scanning

This study was conducted during July 2014. Five fillies of 18-24 months and weighing 180-250 kg were used. The fillies were kept in an indoor paddock and were maintained on a commercial pelleted ration and hay with free access to water. Fillies were kept under the natural day light and temperature. A Pubertal Colt was kept with fillies in the same paddock to keep females cycle normally and to confirm estrus signs.

The ultrasound scanner was equipped with an auto adaptive linear-array transrectal transducer (sonovetR3, Medison, Samsung, South Korea) for the examination of ovaries and uterus, performed as described previously (Ginther, 1995). The experimental period of 18 days extended from July 10 to July 28. Fillies were examined at least twice before conducting the experiment to confirm ovulation.

Follicles

On each day of the 18-day period, all follicles per ovary were measured with the electronic calipers of the ultrasound and their number was counted. 1st (dominant), 2nd (subordinate) and 3rd large follicles were subjected to Day-to-day identity in addition to mapping their location on the same ovary. Day of ovulation (day 0) is the last day the 1st large follicle monitored and not scanned again after that. The disappearance of both uterine edema and signs of estrus confirmed ovulation. Follicular wave were detected and characterized for 6 days before ovulation according to (Ginther and Bergfelt, 1992).

Statistical analysis

Analysis of variance (ANOVA) was used for sequential data to identify the growth rate of all classes of follicles during the estrus phase. The estrus phase starts from six days before of ovulation (day -6) till day of destined ovulation (day 0). Data of figures are presented as mean ±standard error of mean (SE). Tukey HSD and Tukey B tests were performed to differentiate between significant means. Data was processed using SPSS (version 16, 2007).

3. RESULTS

Data analysis revealed that mean number of small, medium, large and total follicles was not significantly changes from six days before ovulation till day of ovulation (day 0).

It is obvious from table (1) that mean number of small follicles increased two days (day -2) before ovulation (day 0). Mean number of medium follicles was similar during days -3,-5, and -6 and was lowest on two days before ovulation. Compared to day 0, number of large follicles was significantly high on day -6. A significantly low mean number of large follicles was observed on the day before ovulation.

Seven days before ovulation diameter of the follicle destined to ovulate (dominant, ovulatory) exceeded 25mm and persisted at this diameter for two days in a plateau then increased gradually to reach its maximum diameter the day before ovulation and the day of ovulation (Table 2).
Table (1): Mean ±SEM (standard error of mean) of number of small, medium, large and total follicles during preovulation from day -6 before ovulation till the day of ovulation (day 0)

<table>
<thead>
<tr>
<th>Days</th>
<th>Small</th>
<th>Medium</th>
<th>Large</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>-6</td>
<td>4.67±3.18</td>
<td>2.00±0.58</td>
<td>2.67b±0.33</td>
<td>9.00±2.31</td>
</tr>
<tr>
<td>-5</td>
<td>5.67±1.76</td>
<td>2.00±0.15</td>
<td>1.67ab±0.33</td>
<td>8.00±2.08</td>
</tr>
<tr>
<td>-4</td>
<td>6.80±1.74</td>
<td>1.50±0.29</td>
<td>1.60ab±0.40</td>
<td>9.60±1.89</td>
</tr>
<tr>
<td>-3</td>
<td>5.17±1.28</td>
<td>2.00±0.58</td>
<td>1.40ab±0.25</td>
<td>7.33±1.33</td>
</tr>
<tr>
<td>-2</td>
<td>7.00±0.89</td>
<td>1.00±0.00</td>
<td>1.50ab±0.29</td>
<td>8.80±0.97</td>
</tr>
<tr>
<td>-1</td>
<td>6.64±0.89</td>
<td>1.50±0.50</td>
<td>1.20ab±0.20</td>
<td>7.86±0.70</td>
</tr>
<tr>
<td>0</td>
<td>6.50±0.96</td>
<td>1.20±0.20</td>
<td>1.38ab±0.26</td>
<td>8.20±0.93</td>
</tr>
</tbody>
</table>

Mean with different superscripts are significantly different at P<0.05

The minimum diameter of small follicles increased on days 4 and 1 before ovulation but the maximum small follicle diameter increased on days -6, -4 and -1 (Figure 1). Contrary, minimum diameter of medium follicle was highest on day -4 then decrease in diameter till day -1 and re-increase again in diameter on day 0, but the maximum medium follicle diameter was observed on day -5 before ovulation and decrease linearly till -2 before ovulation then re-increase again till day 0 (Figure 1).

The two large follicles follow the same pattern of growth from day -6 till day of ovulation (Figure 3). After deviation to dominance, the ovulatory follicle starts growth from 25mm on day -6 till exceed 36mm in diameter day 0.

The difference among diameter of first large follicle, second, third large follicle and the maximum diameter of medium follicle on day 6 before ovulation is about 5.6 and 11 mm, indicating that deviation and dominance starts several days before. The increase in the difference between the three large follicles and the medium one indicated that selection of a follicle to ovulate depends on the maximum diameter it gain faster than others. The difference between the maximum diameter of the 1st large follicle and the minimum diameter of the 2nd large follicle is 11.2 mm.

Table (2): Variation in the mean diameter of First large (dominant, ovulatory): follicle during the estrous phase

<table>
<thead>
<tr>
<th>Days</th>
<th>Number</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Std. Error</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>-6</td>
<td>3</td>
<td>28.13a</td>
<td>5.62</td>
<td>3.25</td>
<td>21.70</td>
<td>32.10</td>
</tr>
<tr>
<td>-5</td>
<td>3</td>
<td>28.23a</td>
<td>4.25</td>
<td>2.46</td>
<td>23.90</td>
<td>32.40</td>
</tr>
<tr>
<td>-4</td>
<td>5</td>
<td>31.18ab</td>
<td>4.80</td>
<td>2.15</td>
<td>24.10</td>
<td>36.10</td>
</tr>
<tr>
<td>-3</td>
<td>5</td>
<td>30.94ab</td>
<td>5.80</td>
<td>2.59</td>
<td>25.00</td>
<td>38.50</td>
</tr>
<tr>
<td>-2</td>
<td>4</td>
<td>29.48ab</td>
<td>4.07</td>
<td>2.04</td>
<td>26.70</td>
<td>35.40</td>
</tr>
<tr>
<td>-1</td>
<td>5</td>
<td>36.50ab</td>
<td>2.89</td>
<td>1.29</td>
<td>33.10</td>
<td>41.00</td>
</tr>
<tr>
<td>0</td>
<td>8</td>
<td>35.54ab</td>
<td>6.34198</td>
<td>2.24</td>
<td>22.00</td>
<td>42.30</td>
</tr>
<tr>
<td>Total</td>
<td>33</td>
<td>32.26</td>
<td>5.62</td>
<td>0.98</td>
<td>21.70</td>
<td>42.30</td>
</tr>
</tbody>
</table>

Mean with different superscripts are significantly different at P<0.05
Figure (1): mean ± SE (Error bars) of minimum and maximum diameters of small follicles.

Figure (2): mean ± SE (Error bars) of minimum and maximum diameters of medium follicles.

Figure (3): mean ± SE (Error bars) of minimum and maximum diameters of large follicles.
4. DISCUSSION

The small pre-ovulatory follicle size is associated with low oocyte viability, then this may account, at least in part, for the poor fertility rates characteristic of mares (Morel et al., 2010). In fillies of this study, the mean difference between the diameters of first two large follicles on day 6 before ovulation is 6 mm but when difference based on the maximum diameter of the 1st and the minimum diameter of the 2nd large it was 11.2 mm. Compared to adult mares, dominance of the largest follicle was indicated by a wide difference of mean 18 mm in maximum follicle size relative to the second largest follicle (Bergfelt and Ginther 1993). Pubertal mares of this study had lower number of follicles per ovulatory waves which are similar to Miniature ponies that had fewer growing follicles ≥10 mm per ovulatory wave and more ovulatory waves with only one follicle ≥10 mm than large ponies and horses (Gastal et al., 2008). Maximum diameter of the preovulatory follicle was similar to that previously observed in mares (Buratini et al., 1997), but Miniature ponies had smaller periovulatory follicle diameter (38.3±0.7 mm) than in horses (44.5±1.4 mm; Gastal et al., 2008). The mean maximum diameter of the dominant follicle of pubertal fillies was nearly similar to that recorded for Mangalarga mares (Buratini et al., 1997), that was 39.0±3.9 mm during one and was 34.7±2.5 mm during two major follicular waves per cycle but was higher than maximum diameter of the dominant follicle present in the secondary wave that was 34.3±11.0 mm (Buratini et al., 1997). The growth of the ovulatory follicle recorded in fillies is similar to that recorded in Lusitano mares from 7 days before ovulation (Mata, 2012) which started from 22.5mm day 7 before ovulation to reach 45 mm 24 hour before ovulation. Similarly, the preovulatory follicle reaches an average size of 40 mm (Aurich, 2011).

Moreover, season and presence of more than one ovulatory follicle negatively decrease diameter of the first ovulatory follicle (Morel et al., 2010). In prepubertal and ovulating ponies, the largest follicle diameter during the 30-day period of the study was larger in the ovulating group than in the prepubertal group, but the diameter of the second largest follicle did not differ between groups. The daily difference between the largest and second largest follicles was higher for ovulating than prepubertal, also was the difference between the largest follicle diameters minus the diameter of the second largest follicle (Nogueira, 2004). The mean diameter of the largest follicles was significantly larger in ovulatory fillies (38 ± 1 mm) than in prepubertal (26 ± 2 mm) but there was no difference between groups in the size of the second largest follicle. There were more small follicles (<24 mm) in the prepubertal than in the ovulatory pony fillies group, but prepubertal fillies had a smaller number of follicles>29 mm than the ovulatory fillies (Nogueira 2004).

In addition, the maximal diameter of the dominant follicle for secondary waves was significantly smaller than for primary waves and maximal diameter was reached earlier (mean maximal diameter of largest follicle: 37.2±1.0 and 45.8±1.0 roan, (Ginther 1993). Compared to pubertal fillies, the older the mares is the more likely to ovulate from smaller follicles. Overall average ovulatory follicle diameter was 39.95 ± 4.84 mm (range 22-50 mm). Moreover, age affected significantly follicular activity included a group effect for the number of 16–20 mm follicles and day × group interactions for the diameter of the largest follicle and number of 11–15 mm follicles (Carnevale et al., 1993). The growth rate of the ovulatory follicle for 5 days prior to ovulation was different (P<0.0003) among groups; young mares had a faster rate of follicular growth than older mares (15 or more years of age) (Carnevale et al., 1993). A reciprocal temporal association existed between the decrease in mean number of >15 mm follicles and the increase in mean numbers of 16–20 mm and > 20 mm follicles. A decrease was observed in the mean number of > 20 mm follicles and in the mean diameter of the second largest follicle between Day-7 to Day-1 with a continuous increase in number of follicles greater than 10 mm until Day-6 or Day-7 when the follicle destined to ovulate was selected (Pierson and Ginther, 1987).

In addition to the breed difference, age (Carnevale et al., 1993), season (Pierson and Ginther, 1987), number of waves during the interovulatory interval(Ginther, 1993 , Buratini et al., 1997) and the presence of more than one ovulatory follicle (Morel et al., 2010) all decrease the growth rate and the maximum diameter of the ovulatory follicles in addition to their effects on follicle populations.

Conclusion

Ovulatory follicle diameter and growth is similar to that of older mares of the same breed.
Studying follicular dynamics in younger ovulatory fillies allowed

5. REFERENCES


