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Follicular, Luteal and Uterine Hemodynamics before and after Dominant Follicle Aspiration in Aged Mares

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Abstract

To study the effect of follicle aspiration on the follicular, luteal, and uterine dynamics and hemodynamics associated with estradiol and nitric oxide changes, five aged mares >18 years were granted from Police department due to aging were subjected to transvaginal dominant follicle aspiration (TVA) on Day 12 after ovulation (Day 0). Before TVA, three complete estrous cycles were studied. The three large follicles were tracked after spontaneous ovulation and follicle aspiration starting from the day of aspiration (Day 0) till Day 12 and Day 15 of the first and second estrous cycles following aspiration. Follicle number, diameter, antral diameter, area, antral area, color area, color area %, granulosa area, and granulosa color area % in relation to the diameter of the dominant and subordinate follicles were determined during the spontaneous estrous cycles. Follicle diameter, antral diameter, area, antral area, color area, color area %, granulosa area, granulosa color area %, CL color area % and uterine horns color area % in relation to days before or after follicle aspiration were determined during the spontaneous estrous cycles. Blood samples collected on the same day of ultrasound and Doppler assessment were used to assay estradiol and nitric oxide concentrations. Days after ovulation influenced Uterine horn area ($P<0.05$) and color area ($P<0.05$), CL color area ($P< 0.05$) and CL color area % ($P<0.05$), Estradiol (E2, $P<0.0001$), and Nitric oxide (NO, $P<0.0001$). Days after aspiration influenced follicle antrum ($P<0.01$), follicle circulatory ($P <0.0001$), CL diameter ($P<0.05$), CL area ($P<0.05$), and E2 ($P=0.007$). Aspiration influenced the follicle circulatory ($P<0.0001$) and CL color area ($P<0.05$) and tended to influence E2 ($P>0.05$). In conclusion, aspiration of the dominant follicle simulates spontaneous ovulation in aged mares. Day 12 after ovulation could be selected for aspirating the dominant follicle without disturbing the follicular dynamics and hemodynamics. Aspirated dominant follicle become a corpus luteum with 2-3 days after aspiration.

KEYWORDS
Dominant follicle, Aspiration, Hemodynamics, Nitric oxide, Estradiol hormone, Mares

INTRODUCTION

Follicular dynamics in mares is very similar to women, so mares were used as a large animal model to study ovarian follicular dynamics (Adams *et al*., 2012) and follicular waves in women (Ginther *et al*., 2004a; Ginther, 2012). Because of the similarity between mares and women, mares were used for studying the effect of aging and obesity on ovarian dynamics and used as assisted reproduction model for many reasons among them is the ethical concerns in using subjects (Benammar *et al*., 2021). In contrast to women, ovulation stops in 17% of mares reached 20 years (Vanderwall *et al*., 1993; Carnevale *et al*., 1994) and continued until 25 years in others (Ginther, 1992).

In mares, the first applications of follicle aspiration or ablation was for studying follicle selection and deviation (Gastal *et al*., 1997). Dominant follicles (Schauer *et al*., 2013; Ignácio *et al*., 2021), immature follicles (Gastal *et al*., 1997; Velez *et al*., 2012) were aspirated several times from the same mare during one breeding season for studying the ovarian follicular dynamics, dominant follicle selection and deviation.

Follicle aspiration for collecting oocytes was recommended for younger sport, infertile (Carnevale *et al*., 2005), and aged mares (Hendriks *et al*., 2015) though fertility decline with aging (Ginther, 1992; Cuervo-Arango *et al*., 2019). *In vivo* mature preovulatory oocytes (Carnevale, 2004) and immature ones (Choi *et al*., 2002; 2004) were aspirated for the *in vitro* embryo production from transitional, cycling, and pregnant mares (Purcell *et al*., 2007). Both mature and immature follicles aspiration was practiced for oocyte recovery during practice (Rodriguez *et al*., 2021) and commercial OPU/ICSI programs (Claes *et al*., 2016).

Though, ovarian follicle aspiration for oocyte recovery required sedation, epidural anesthesia, and muscle relaxant but studies on the effect of aspiration and oocyte recovery on the mare health, stress, and pain reflexes were performed (Diego *et al*., 2016). Recently, days during estrus (24 h after GnRH stimulation) and after ovulation (Day 7 and Day 14) were studied aimed to aspirate higher number of mature oocytes available for intracytoplasmic sperm injection (ICSI; Walbornn *et al*., 2022). Oocyte aspiration was practiced using long double lumen needle for equine (Carnevale *et al*., 2005) and short disposable needle

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system (Lee *et al*., 2015). Effect of repeated follicle -aspiration on ovarian hormones and corpus luteum function was studied (Iacono *et al*., 2014) for identifying their effects on chromatin and cytoplasmic characteristics (Vernunft *et al*., 2013). Preantral follicles were aspirated for histological evaluation and validation the use of scalpel for mechanical recovery from slaughterhouse collected ovaries (Haag *et al*., 2013)

The assessment of the corpus luteum function by assaying progesterone secretion and hemodynamics played important role in the selection on the next dominant follicles even from before their deviation (Ginther, 2019).

This study aimed to understanding the effects of transvaginal dominant follicle aspiration (TVA) of the dominant follicle for recovering higher number of oocytes from aged mares, determine the suitable timing after aspirating for repeating the procedure, to compare the follicular dynamics after ovulation and after aspiration in association with circulating estradiol and nitric oxide concentrations, and to help veterinarians to maximize the efficiency of this procedure in aged infertile Egyptian horses for future oocyte recovery and *in vitro* embryo production.

MATERIALS AND METHODS

Animals and location

Before starting this study, the animal care and use committee of Faculty of veterinary Medicine, Cairo University has approved the protocol (Vet-CU- 01122022586). Mares (n.=5) of >18 years old and of body weight from 400-600 kg of different breeds were kept in the experimental farm of the Theriogenology department, Faculty of Veterinary Medicine, Cairo University. Mares were kept under the natural day light and temperature and fed two times per day on ration fulfilling their maintenance nutritional requirements. The study started from March 2022, stopped during the hot summer months (May to August), and resumed to January 2023.

Ultrasound examination

The ultrasound-Doppler scanner (SONOACE R3, Samsung Healthcare, South Korea) equipped with 12 MHz linear-array trans-rectal transducer was used for the examination of ovaries with ovarian arteries in addition to uterus with uterine arteries as previously described (Ginther, 1995). The right and left ovaries were scanned on each other day in respect to the follicles of each ovary and their hemodynamics. Ovarian follicles dynamics and hemodynamics were studied for three complete estrous cycle before follicles aspiration where Day 0 is the day of ovulation and for 12 or 15 days after first and second dominant follicle aspiration where Day 0 is the day of aspiration. The endo-cavitary 8 MHz Micro-convex transducer with a home-made probe holder was used for ovum pick up procedure. Before follicle aspiration, the day when at least one large follicle diameter achieved 20 mm was selected for follicle aspiration. Blood flow vascularization of the large three follicles was determined after ovulation and each other day from Day 1 to day 15 after TVA using colored doppler. The corpus luteum dynamics and hemodynamics were studied for the complete estrous cycles after ovulation and after dominant follicles aspiration. Ovarian follicles were classified according to their diameter into Dominant (F1, the largest follicle diameter at any day of the estrous cycle and >3.0 cm during the preovulatory phase); subordinate 1 (The second large follicle following F1 in diameter and its diameter is > 2.0 to < 3.0 cm in diameter; and subordinate 2 (The third large follicle of diameter >1.0 and < 2.0 cm).

Follicle aspiration Technique

For aspirating the follicular fluids from dominant follicle, mares were scanned with ultrasound for three successive estrous cycles to determine the dominant pre-ovulatory follicle diameter (>35mm). For collecting the immature oocytes, the donor mares were scanned transrectally by ultrasound from one to three days before the day of aspiration to evaluate the number and size of the follicles present on the ovaries, considering the presence of at least eight to ten follicles on both ovaries of >1 cm of diameter. The endo-cavitary 8 MHz Micro-convex transducer with a home-made holder (designed to keep probe) and double-lumen equine follicle aspiration needle were used for the follicle aspiration to keep monitoring each follicle to be aspirated to see follicles shrink on aspiration and to direct the needle for follicle puncture. Ovarian follicles flush (Equine Euro-flush media, Minitube, Germany) was prepared in animals used for ovum pick up. For the complete aspiration of each follicle, a negative pressure 200Hg was induced using suction pump (William A. Cook, Brisbane, Australia).

Preparation of Mares for TVA

A urine catheter was used and a collection bucket for collecting urine before starting the anesthesia. Mares were sedated with Xylazine HCl (1 mg/Kg IV) three times or more during the procedure as required to have a good sedation. A posterior epidural anesthesia (4-8 mL of 2% lidocaine) was also applied just before follicles aspiration. Antral follicles were punctured using a 12G double lumen needle (Minitube, Germany) mounted with the home-made needle guide on a modified native endo-vaginal probe holder and repeatedly flushed several times with follicle flushing media supplemented (Euroflush, Minitube, Germany) supplemented with 5 IU/mL of heparin (Heparin-Natrium 5000 UI/mL), to prevent clotting of follicular fluid and blood (Galli *et al*., 2014). The probe in probe holder was covered by a disposable sterilized latex (Director Guideline verification kit, Protek Medical Products, 4125 Westcor Court, Coralville, IA 52241, United States). The double lumen needle was connected to the bottle receiving follicular fluid and flushing media using a specialized disposable tubing system for equine oocyte follicle aspiration (Minitube, Germany). The suction pump is also connected to the collection bottle using a tubing system with filter to prevent the entrance of any fluids into the suction pump (Cook, Australia).

The candidate ovary was fixed by the left hand and the designed follicle to be aspirated was fixed in front of the apex of the transducer. The home-made probe holder keeping the endo-vaginal transducer fixed is covered by the sterilized lattices is lubricated by the sterilized gel and is introduced into the vagina by the right hand. Once the follicle is monitored, the monitor in inverted and the biopsy line is activated. After activating the biopsy line guide, the double lumen needle was advanced in the needle holder then aspiration started by starting the negative pressure by the foot twitch using the right leg. The follicle was flushed using a syringe connected to the second lumen of the equine double lumen biopsy needle. Both the follicle flushing media and the collection bottle are kept in water bath at the body temperature of the mare.

The recovered flushed fluid was filtered with an embryo filter (EmSafe Embryo Filter, Minitube, Germany) and the same filter was used as searching dish to search for any oocytes under Stereo microscope.

Blood sampling, hormonal and nitric oxide measurements

Blood samples (5 ml) were collected via the jugular vein with each ultrasound examination in either plain or EDTA (5 ml) vacutainer tubes. The obtained sera and plasma samples were stored at -20°C till hormone assay. Concentration of serum estradiol (E2) was measured using ELISA commercial kits. Nitric oxide metabolites (NOMs) were analyzed in blood sera (Biodiagnostics, Egypt).

Image analysis

For determining the quality of follicle, the digital video recording was exported from the hard disk of the ultrasound machine to a computer by digital data traveler. The good images of ovarian structures with B- and color modes were processed using image analysis software (Adobe Photoshop software (1990–2013, Adobe Systems) for further digital image analysis. The granulosa layer was calculated by subtracting the area of the follicle antrum (Pixel) from the follicle area (Pixel). The granulosa color area percent was calculated by dividing the color area within the follicle granulosa (Pixel) on the granulosa area (Pixel). The follicle color area percent was calculated by dividing the color area within the follicle (Pixel) on the follicle area (Pixel). The corpus luteum color area percent was calculated by dividing the color area (Pixel) within the corpus luteum (CL) on the total CL area (Pixel). The uterine horns color area percent was calculated by dividing the color area within the uterine horns (Pixel) on the uterine horn area (Pixel).

Statistical analysis

The data are presented as means ± standard error of the mean (SEM). Analysis of variance (ANOVA) was used for studying the effect of day of the estrous cycle before or after follicle aspiration where day 0 is the day of ovulation in cycles without aspiration and the day of aspiration (Day 0) in cycles with aspiration on the studied parameters. For comparing parameters on each day of the estrous cycle before follicle aspiration and after follicle aspiration (Days 0, 1, 3, 5, 7, 9, 10, 12, 15). Independent sample student t-test was used. Data are presented in figures with error bars where $P < 0.05$ is considered significant. Simple one-way ANOVA was also used to study the effect of follicle type (dominant, subordinate 1, and subordinate 2) within different days of the estrous cycle.

RESULTS

The follicular population number (Fig. 1A) did not vary throughout the days of the spontaneous estrous cycle. The number of total follicles was the same on Days 10 and 12 after ovulation or after aspiration (Fig. 4). The number of dominant and

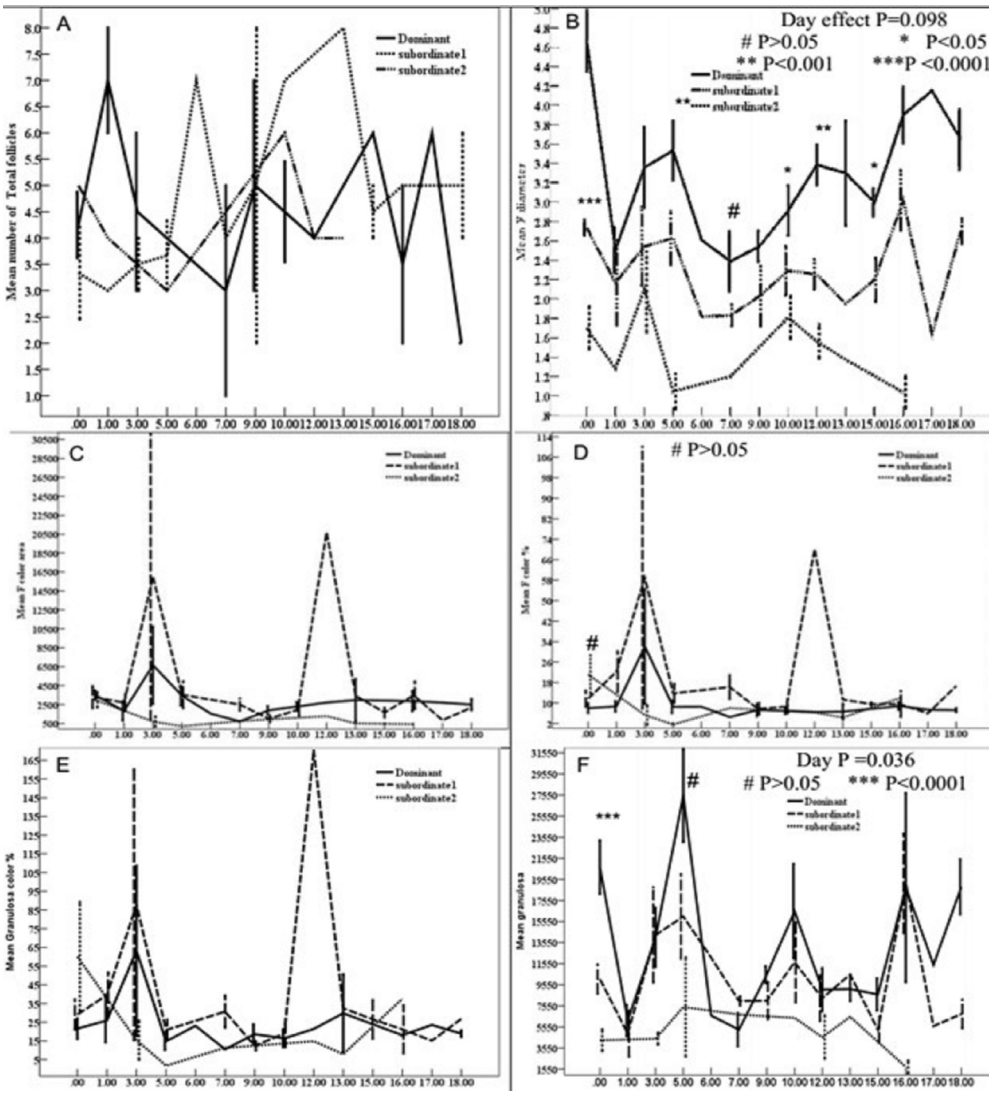

Fig. 1. Mean number of total follicles (A); Follicle diameter (B), Follicle color area (C); Follicle color area percent (D); granulosa color area % (E); granulosa area (F) of dominant and subordinate follicles throughout the days of the estrous cycle where the Day of ovulation (Day 0) with error bars.

Table 1. differences in follicular dynamics, hemodynamics uterine horn and luteal hemodynamics hemodynamics in relation to follicle class, effect of dominant follicle (F1) aspiration, and days before and after F1 aspiration

Parameter	Dominant	Subordinate 1	Subordinate 2	P-value	aspiration	Days after	
						Ovulation	Aspiration
Total follicles	4.22±1.83	4.45±1.82	4.33±1.73	0.9	NS	NS	NS
F. diameter /cm	3.42±.99[c]	2.39±.69[b]	1.56±.52[a]	0.00	NS	NS	NS
Antrum diameter /cm	2.45±.91[c]	1.71±.61[b]	1.21±.52[a]	0.00	NS	NS	0.01
F. circularity %	21.99±33.44[a]	28.16±35.45[a]	52.35±32.17[b]	0.00	0.00	NS	0.00
F. area /pixel	35484±17285[c]	21324±12612[b]	9870±5376[a]	0.00	NS	NS	NS
Antrum area /pixel	19040±11976[b]	10392±8169[a]	5795±4743[a]	0.00	NS	NS	NS
Granulosa area /pixel	15279±9184[c]	10787±7657[b]	4782±2877[a]	0.00	NS	NS	NS
F. color area /pixel	3049±2881	3816±6001	1364±1369	NS	NS	NS	NS
F. color area %	10.05±14.62	16.85±20.86	10.65±9.99	NS	NS	NS	NS
Granulosa color area %	24.09±30.58	32.99±36.97	28.46±33.91	NS	NS	NS	NS
Ut H diameter /cm	2.93±.63	2.91±.56	3.322±.61	NS	NS	NS	NS
Ut. H. area/pixel	32916±11686[a]	30835±9328[a]	48387±30106[b]	0.05	NS	0.03	NS
Ut. H. color area /pixel	2301±1085	2686±1612	3131±1016	NS	NS	NS	NS
Uterine color area %	6.63±2.17	9.14±4.71	9.41±2.85	NS	NS	0.02	NS
CL diameter /cm	2.64±.71	2.92±.87	2.63±.96	NS	NS	NS	0.04
CL area /pixel	28466±10763	31133±15422	37369±5071	NS	NS	NS	0.04
CL color area	5358±4358	4648±2941	7630±4903	NS	0.04	0.02	NS
CL color area %	21.21±8.07	13.87±8.47	21.06±14.79	NS	NS	0.05	NS
E2 pg/ml	51.09±37.35	56.34±44.03	53.68±42.61	NS	0.07	0.00	0.01
NO µmol/L	20.25±4.40	20.18±4.59	21.09±2.14	NS	NS	0.00	NS

Data are expressed as Mean± SD. F: follicle; F1: dominant follicle; Ut. H.: Uterine horn; CL: corpus luteum; NS: non-significant.

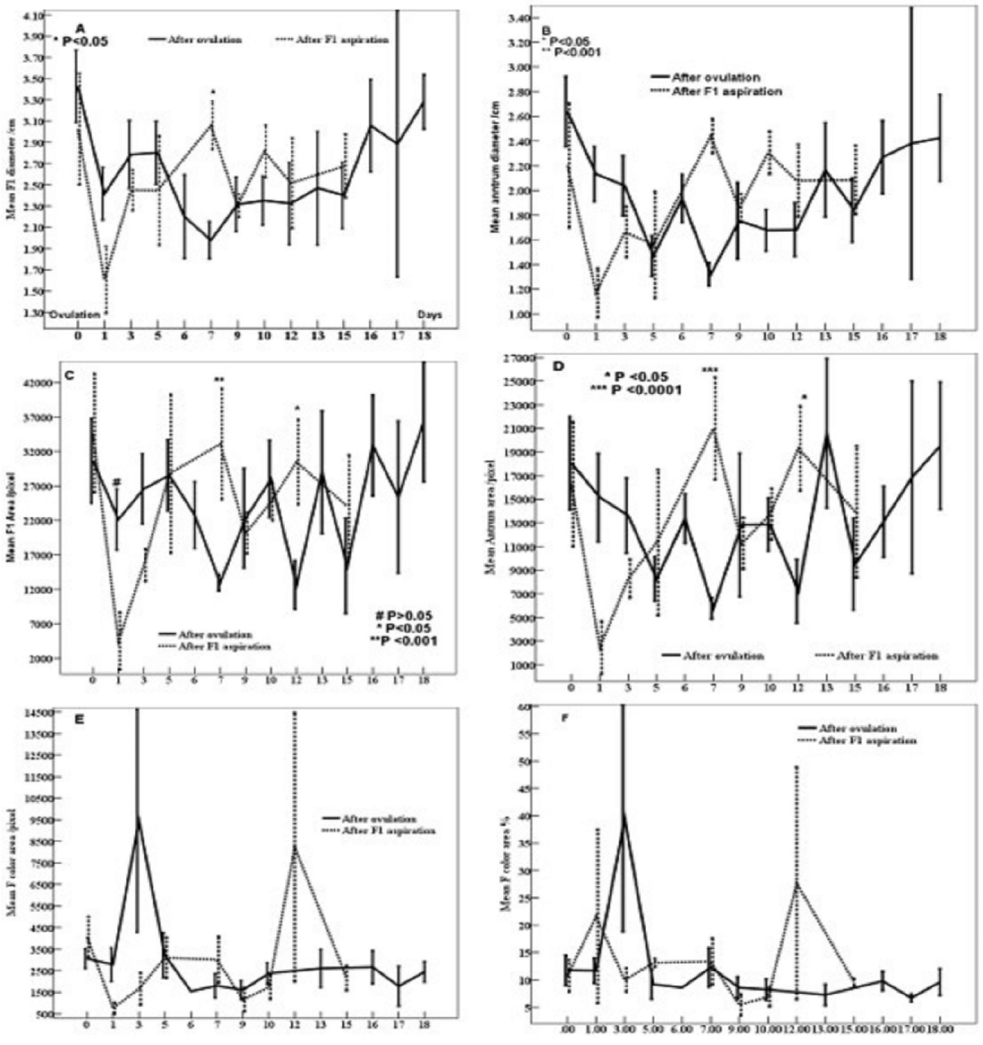

Fig. 2. Mean follicle diameter /cm (A); Follicle antrum diameter /cm (B), Follicle area / pixel (C); Follicle antral area / pixel (D); follicle color area /pixel (E); follicle color area % (F) of before and after follicle aspiration throughout the days of the estrous cycle where the Day of ovulation and aspiration (Day 0) with error bars.

subordinate follicles was maximum from day 10 to day 15 after ovulation. The diameter of the dominant and subordinate follicles varied (P<0.0001) on the day of ovulation (Day 0; Fig. 1B) where the dominant follicle diameter reached >4.5 cm, the first subordinate (subordinate 1) reached 2.8 cm and the second subordinate (subordinate 2) did not exceed 2 cm in diameter. On Day 7 (P>0.05), Day 10 (P<0.05); Day 12 (P<0.001); and Day 15 (P<0.05) the difference in the diameters of the dominant is the highest diameter compared to the subordinate follicles (Fig. 1B). The follicles color area (Fig. 1C), the follicles color area % (Fig. 1 D), and the follicle granulosa color area % (Fig. 1E) of the dominant follicle were lower than the subordinate1 on days 3 and 12. The dominant follicle granulosa area (Fig.1 F) showed significant increase on Day 1(P<0.0001), Day 5 (P>0.05) and Day 10 compared to the subordinate follicle.

Follicle diameter, antral diameter, area, antral area, and granulosa area linearly declined (P<0.0001) from the dominant, subordinate1, to reach the lowest one for the subordinate 2 (Table 1). The increase of the diameter of the dominant follicle is associated with a decrease in its circulatory % (P<0.01). Aspiration of the dominant follicle (Table 1) influenced the circulatory percent (P<0.0001) and the corpus luteum color area (P<0.05).

Days of the estrous cycle influenced the uterine horn color area (P<0.05) the uterine horn color area % (P<0.05), CL color area (P<0.05), CL color area % (P=0.05), the concentrations of the estradiol (E2; P<0.0001), and nitric oxide levels (P<0.0001; Table 1). Days after F1 aspiration influenced follicles antrum area (P<0.5), follicle circulatory (P<0.0001), CL diameter and area (P<0.05), and estradiol concentrations (P<0.01). Follicle diameter increased on Day 7 after aspiration compared to Day7 after ovulation (Fig. 2A). Follicle antral diameter sharply decreased on Day 1 after aspiration (P<0.05) and increased on Day 7 (P<0.001) and Day 10 (P<0.05) compared to the corresponding days after ovulation (Fig. 2B). The follicle area declined (P>0.05) on Day 1 but increased on Day 7 (P<0.001) and Day 12 (P<0.05) after aspiration compared to the same days after ovulation (Fig. 2C). The follicle antral area increased on Day 7 (P<0.0001) and Day 12 (P<0.05) after aspiration compared to days 7 and 12 after ovulation (Fig. 2D). The follicle granulosa area declined (P<0.05) on Day 1 but increased on Day 7 (P<0.05) and Day 12 (P<0.05) after aspiration compared to these days after ovulation (Fig. 3a). The follicle granulosa color area % (Fig. 3b) and the CL color area % (Fig. 3c) increased on Day 5 (P<0.05) after aspiration compared to after ovulation. The uterine horn color area % declined (P<0.05) on Day 9 after aspiration (Fig. 3d).

DISCUSSION

The follicular dynamics was studied for three estrous cycles to select the suitable day for future oocyte recovery. It is evident that days from day 10 to day 15 after ovulation can be used for aspirating dominant follicle. In agreement with the investigated mares, total follicle numbers differed significantly between individual mares but did not differ following ovulation or dominant follicle aspiration where ovum pick up was performed for aspirat-

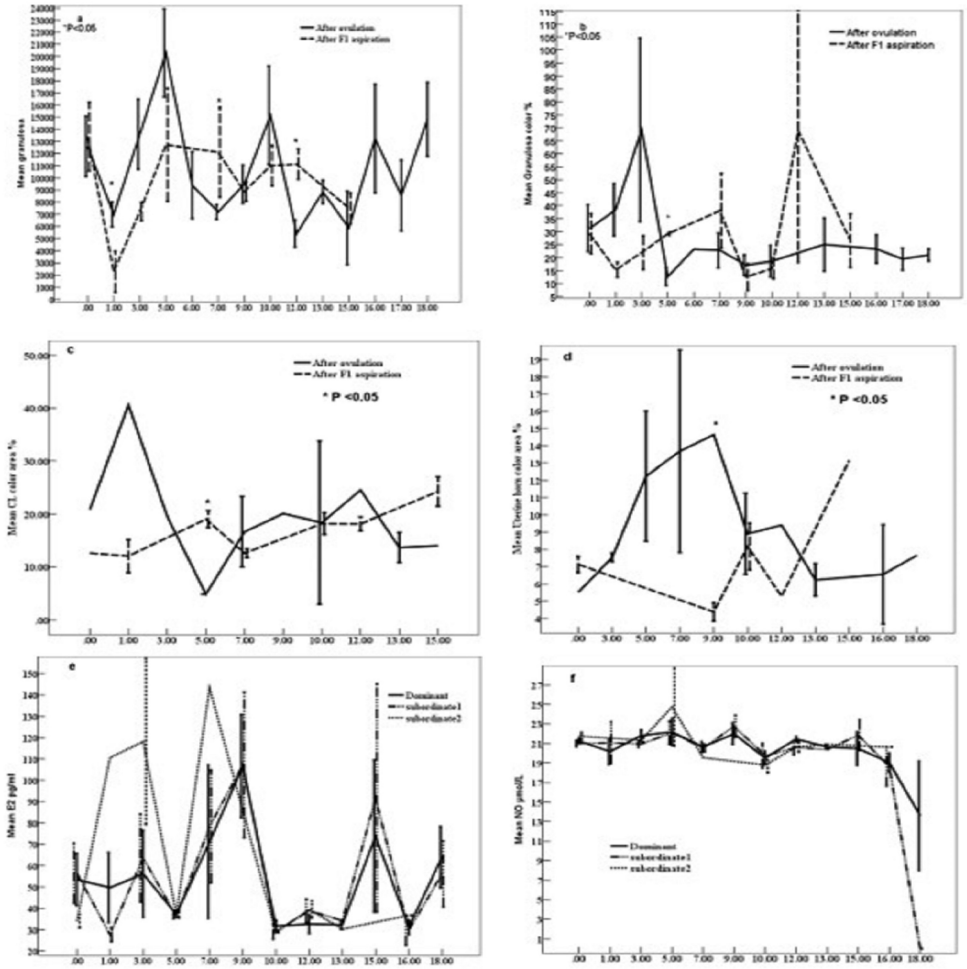

Fig. 3. Mean Follicle granulosa area/ pixel (a), Follicle granulosa color area % (b); CL color area percent (c); uterine horn color area % (d) before and after follicle aspiration throughout the days of the estrous cycle where the Day of ovulation and aspiration (Day 0) with error bars. Mean estradiol pg/ml (e); nitric oxide (NO µmol/L; e) during the growth of dominant and subordinate follicles throughout the days of the estrous cycle where the Day of ovulation (Day 0) with error bars.

ing oocytes between Day 7 and Day 14 after ovulation (Walbornn *et al*., 2022). In this study, the tracked three large follicles numbers after ovulation did not vary significantly at any day after ovulation, but more subordinate follicles and fewer dominant follicles were recorded on Day 7 and more dominant follicles were noticed on Day 15 after ovulation. Similarly, small follicles <10mm increased on Day 7 and fewer follicles >20 mm were recorded on Day 14 days after ovulation and during estrus (Walbornn *et al*., 2022). In agreement with the obtained results, the deviation of the dominant and subordinate1 follicle of Criollo breed mare started on Day 7 where the dominant follicle started deviation to exceed the diameter of 25mm and the subordinate follicle have a diameter <22mm (Duval *et al*., 2022).

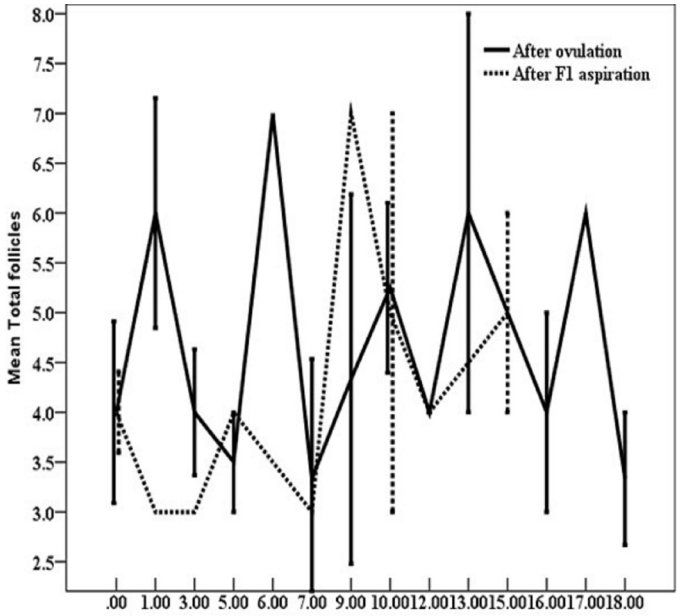

Fig.4. The difference in the follicles number throughout the days of the estrous cycle where the Day of ovulation and aspiration (Day 0) with error bars.

Similar to the decrease in the diameter of the incompletely aspirated follicle performed to mares in this study and its low growth for five days thereafter, the aspiration of 20µl of the follicular from small and large follicles on Day 15 from mares resulted in a transient decrease in diameter form 24-48 h and kept slow growth for five days after aspiration to a final diameter lower than those not aspirated which was referred to the leakage of the follicular fluid after aspiration (Gastal *et al.* 1999a).

Aged mares of the current study showed sharp decrease in the follicle diameter after ovulation (Day 1) where the dominant follicle started increase from Day 7 reaching its maximum diameter on Day 12. The dominant follicle reached its maximum diameter on Day 12 and both the subordinate follicles reached their maximum diameter on Day 10. In young mares, the dominant follicle and the three subordinate follicles attained their highest diameter, area, antral area from Day 10 to Day 12 (Abo El-Maaty and Abdelnaby, 2017). The achievement of dominant follicle a diameter >25mm starting from Day 15 in in Criollo breed mares is similar to the increase of the diameter of the dominant follicle on day 15 after aspiration and ovulation in aged mares of this study (Duval *et al*., 2022). In agreement with young mares, diameter, area, granulosa area, and antral area varied significantly with the size of the follicle (Abo El-Maaty and Abdelnaby, 2017). The dominant follicle achieved a diameter of >2.3cm from day 10 after ovulation and follicle aspiration indicating the emergence of the next ovulatory follicular waves in aged mares and on Day 10.5 in younger mares (Ginther, 2012). The dominant follicle attained lower circulatory % with advancement of the time to ovulation compared to the two subordinates in aged mares of the current study. As well as the dominant follicle starts losing its circulatory starting from Day 10 (Abo El-Maaty and Abdelnaby, 2017).

In aged mares of this study, the indifference in the follicle color area, color area % and granulosa color area % of the dominant and the two subordinate follicles of aged mares after ovulation is contradicting their difference with the follicle size in younger mares (Abo El-Maaty and Abdelnaby, 2017). This contrast could be attributed to the study of the three large follicles in aged mares and the two large follicles on either ovary in young mares. In aged mares of this study, the dominant and the subordinate1 had the highest granulosa cells layer on Day 5, 10 and 16. The increase of the subordinate2 follicle color area and color area % on Day 12 is similar to the higher follicles colored pixels % of the three subordinate follicles compared to the preovulatory one from Day 5 to Day 12 (Abo El-Maaty and Abdelnaby, 2017).

The partial aspiration of large follicle resulted in the luteinization of this follicle and the development of a corpus luteum. the CL color area % increased from day 7 to day 9 after ovulation and on day 5 after follicle aspiration. In agreement with aged mares, young mares had the highest CL color area % on days 5 and 6 after ovulation (Abdelnaby and Abo El-Maaty, 2017). The development of corpus luteum at the site of the aspirated follicle in aged mares after follicle aspiration was noticed in mares subjected to follicle aspiration with a diameter >25mm (Mozzaquatro *et al*., 2010). Moreover, repeated ovarian punctures and aspiration did not affect the corpus luteum function or development (Bøgh *et al*., 2003). In agreement with the significant effect of days after ovulation on CL diameter and CL area after aspiration and the significant effect of days after ovulation on CL color area and color area %, young mares showed significant effect of the day after ovulation on CL area, volume, CL vascularization area and CL colored area % increased significantly from Day 1 to Day 11 after ovulation (Abdelnaby and Abo El-Maaty, 2017).

Aged mares of the current study >18 years showed ovarian cyclicity with shorter estrous cycle length. In agreement with results from the present study, mares reach their ovarian senescence on average at 25 years old (Ginther, 1992). Only, <20% of mares older than 20 years stop ovulation (Vanderwall *et al*., 1993; Carnevale *et al*., 1994).

Similar to the increase of estradiol (E2) on days 9 and 15 in dominant and subordinate1 follicles and on Days 3 and 5 during the subordinate 2 growth, concentrations of estradiol increased in selected large follicles was attributed to increased E2 synthesis by upregulating the theca synthesis (Ginther *et al*., 2004 b,c) and by preventing the metabolism of pregnenolone synthesized by granulosa cells to progesterone (Ginther *et al*., 2002). Aspirating the large follicles declined estradiol for two days after aspiration which started to increase with the development of the next ovulatory wave (Gastal *et al*., 1999b). Estradiol increased on the day of follicle deviation or a day earlier (Gastal *et al*., 1999a).

In agreement with young mares, nitric oxide concentrations increased on Day 5 and Day 12 in the presence of all follicle classes (Abo El-Maaty and Abdelnaby, 2017). The increase of estradiol in aged mares on Day 9 and day 15 in aged mares is contradicting its gradual decrease from Day 1 to Day 12 after ovulation in young mares (Abo El-Maaty and Abdelnaby, 2017).

CONCLUSION

The aspiration of the large follicle simulate ovulation in mares indicated by the luteinization of the partially aspirated follicle and its development to a corpus luteum. Aspiration of the dominant follicle can be performed starting from Day 10 to day 12 after ovulation or aspiration. Large follicle aspiration influences follicular dynamics for 5 days after aspiration until the selection of the next dominant and subordinate follicles. Follicle aspiration could be repeated within two weeks without any effect of the follicular dynamics and hemodynamics.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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