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Efficacy of Ovulation Synchronization in Arabian Mares using Ovsynch Program: A Closer Look to its Feasibility

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ABSTRACT

The present study aimed to evaluate the Ovsynch program in Arabian mares compared to ovulation synchronization using progesterone. Twenty cyclic Arabian mares, averaging 5.9 years of age, were assigned for this study. Mares were randomly divided into two groups. The first group (GPG, n=10) received an IM injection of 40µg GnRH agonist at the beginning of the protocol and 250µg PGF2α analog on Day 7. Mares in this group were further assigned into two subgroups, G-PG-G(D9) (n=5, second GnRH agonist by Day 9) and G-PG-G(D12) (n=5, second GnRH by Day 12). The second group (G-P-PG-H(D19), n=10) was treated with oral progesterone for 14 days, 250µg PGF2α analog on Day 9, and 2500 hCG on Day 19. All animals were subjected to daily ultrasonographic examination. All animals were inseminated 24 hrs after the second GnRH (GPG) and hCG [G-P-PG-H(D19)] injections. Mares were bled at specific landmarks of the experiment, and sera were collected for progesterone and estradiol-17β assay. Compared to G-P-PG-H(D19)-treated mares, GPG group mares had longer estrus duration, larger DF, longer GnRH-ovulation, and longer AI-ovulation intervals. The conception rate was significantly lower (10%) for GPG compared to the G-P-PG-H(D19) (50%) groups. It can be concluded that factors like unreadiness of a considerable number of predestined ovulatory follicles for ovulation at the time of second GnRH administration, inordinate ovulation in response to second GnRH, and the long interval between insemination and ovulation are probably the reasons behind the low efficiency of this protocol in the Arabian mares.

Key words: Arabian mare; Ovulation synchronization; ultrasound; Conception.

INTRODUCTION

Arabian horse is an elegant animal that takes over the attention of the folks of the Arabian Peninsula who considered it as a national treasure and a legacy of their ancestors. The uniqueness of social life of the Arabian horse limits its extensive production and reproduction (Dimmick et al. 1993). In temperate zones, Arabian mares cycle year round but conception is higher in summer than winter months (Ali et al. 2014). To maximize the efficiency of breeding practices and ultimately the pregnancy rate, accurate ovulation timing prediction has become a vital component of breeding management of Arabian mare (Warriach et al. 2014). Reduced breeding cycles or inseminations maximize stallion or semen usage, reduce contamination of mares, and reduce costs associated with farm labor (Mesut et al. 2022). On the other hand, healthy mares bred with fertile stallions or good quality semen need one mating or insemination per cycle, however, well-managed breeding programs allow up to 10% rebreeding per cycle (Handler et al. 2006).

A common practice to control the estrus cycle in mares includes the use of PGF2α to shorten the diestrus phase (acting on the mature CL) and/or the estrus phase using ovulation enhancers like gonadotropin releasing hormone (GnRH) or its agonists or human chorionic gonadotropin (hCG) when dominant follicle reaches the size of ≥35mm (McCue et al. 2007). Estrus/ovulation synchronization is used widely in most animal species- but horse- to promote their reproductive performance (Yurdaydin et al. 1993). Factors like age, breed, environment, genetic traits and the uniqueness of the estrus cycle with difficult to predict time of ovulation are considered obstacles for uniform synchronization in mares (Dimmick et al. 1993).

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GnRH or its agonist exerts its action via the hypothalamic-hypophyseal portal circulation to induce the secretion of pituitary gonadotropins and consequently the final maturation and ovulation of the ovarian DF (Ginther et al. 2004). Both GnRH and hCG are excellent ovulation inducers in equine practice, however, hCG is favored by many practitioners due to its impact on the oocyte crop in mares (Pastorello et al. 2022). A majority of mares treated with 2000-5000 IU hCG had ovulation within 36-48 hrs following the treatment of dominant follicles greater than 35 mm in mares (Samper 2008). Consequently, fewer matings/inseminations are required per estrus, causing conception rates to improve as synchrony between ovulation and mating increases (Warriach et al. 2014). It was reported that 83.3% and 100% of hCG-treated mares with a follicle greater than 30 mm in diameter ovulate within 48 and 96 hrs, respectively (Mesut et al. 2022).

A combination of more than one hormone has been used to synchronize estrus including progesterone-estrogen (Dos Reis et al. 2020), progesterone-PGF2α and progesterone-hCG (Zielinski et al. 2021) and PGF2α-PGF2α-hCG (Kuhl et al. 2017) with variable outcomes depending on the breed, altitude and season. The use of progesterone is based mainly on its inhibitory action on the hypothalamic-hypophyseal axis during the period of treatment (Ginther et al. 2004). Addition of either estrogen or prostaglandin aims mainly to inhibit the naturally-produced progesterone from the luteal structures in the ovaries to ensure a uniformly-synchronized estrus (Dos Reis et al. 2020). While, GnRH or hCG are used to induce ovulation and oblige the shortening of the estrus duration in the synchronized animals (Bucca and Carli 2011; Kareskoski et al. 2019). Previous work on these combinations pointed out to a variable success rate ranging from 60-90% for ovulation synchronization and 25-80% for conception (Karam et al. 2017).

The use of a combination of GnRH-PGF2α-GnRH (Ovsynch program) never applied in equine practice to synchronize ovulation despite its familiarity in a wide range of domestic animals especially cattle (Nowicki et al. 2017). The reasons behind this abstention can be justified on the basis of variability of the length of the estrus in mares compared with other species (Warriach et al. 2014). Arabian mares had a longer mean interval from Day 0 to ovulation, a smaller follicle prior to ovulation and longer estrus compared to other breeds of horse (Dimmick et al. 1993; Ali et al. 2014; Warriach et al. 2014). However, there is no ground research explains -on clinical basis- the unfeasibility of this protocol in mares particularly the Arabian ones. Therefore, the aim of the present study is to evaluate the ovulation synchronization using the GPG program compared with synchronization with oral progesterone in Arab mares.

MATERIALS AND METHODS

Compliance with ethical standards

This study was approved by the Animal Care and Welfare Committee, Deanship of Scientific Research, Qassim University, Kingdom of Saudi Arabia (Reference Number 213427).

Animals

Twenty nulliparous, cyclic, clinically and reproductively sound Arabian mares were assigned for this study. Animals aged 4–9year (average 5.9), weighed 350-500kgs (average 415kg), 3-4 (average 3) body condition score (Pallesen et al. 2023) at private farm, Riyadh region, central Saudi Arabia (Latitude 24.7136° N and longitude 46.6753° E). The estimation of body condition score mainly depends on the richness of certain areas of the mares' body such as withers, shoulders, chest and tail using visual inspection and fat reserves. Animals kept in individual paddocks and exercised daily for 2 hours at the early morning and before dusk. Studied mares were fed on balanced ration according to NRC (2007) and watered ad libitum. Before the experiment, animals were examined clinically and gynecologically on a daily basis for one complete cycle to ensure their general and reproductive soundness. During the preliminary examination prior to the application of the synchronization protocols), mares were teased daily during the expected estrus and monitored for normal estrus duration and behavior. The experiment was carried out during the breeding season 2023 (June – August).

Experimental design

As in Fig. 1, mares were divided randomly into two groups and treated simultaneously to avoid discrepancies in response to variation in environmental changes during the study months. The first group (GPG, n=10) received 40 μg GnRH agonist Buserelin acetate (Receptal®, Intervet International B.V., Boxmeer, Holland) at the beginning of the protocol. On Day 7 (Day 0 is the first day of the protocol), PGF2α (250 μg cloprostenol; Estrumate TM, Essex Tierarznei, Germany) was administered IM. Then, this group is further assigned into two subgroups each had 5 mares; G-PG-G(D9) (revived second GnRH on Day 9) and G-PG-G(D12) (revived second GnRH on Day 12).

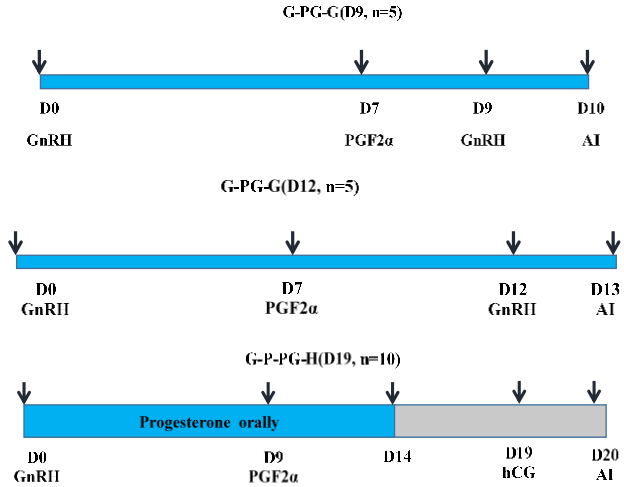

Fig. 1: Experimental design for mares in the study.

The second group (G-P-PG-H(D19), n=10) received daily oral progesterone (regumate, altrenogest Solution 0.22%, 0.044 mg/kg, Intervet Inc, d/b/a Merck Animal Health, Summit, NJ 07901) for 14 successive days and administered PGF2α (250 μg cloprostenol) on Day 9 (Day 0 is the first day of the protocol) and human

chorionic gonadotropin (hCG, 2500 IU Chorulon, Intervet Inc. 126 E. Lincoln Avenue, PO 2000, Rahway, NJ, 07065) on Day 19 (5 days after the stoppage of progesterone treatment). Animals were teased daily to determine the length of estrus.

All animals were inseminated artificially with fresh cooled liquid (insemination dose contains at least 500 x 106 sperms/mL) semen from a fertile stallion) once 24 h following the second GnRH dose for GPG protocol and hCG injection for G-P-PG-H(D19) protocol.

Ultrasound examination

Ovarian changes of all animals were monitored ultrasonographically using a real-time, B-mode, diagnostic scanner equipped with a transrectal 5/7.5MHz linear array transducer (Aloka Co., Ltd., Tokyo, Japan). Ultrasound examinations were performed once daily throughout the protocols until the second GnRH in GPG group and hCG administration in G-P-PG-H(D19) group, and each 12 h thereafter until ovulation. Follicles were categorized into three classes; small (≤20 mm), medium (≥20–30mm) and large (>30 mm). All follicles and corpora lutea (CLs) were measured, and sketched individually. Ovulation was identified when a traced large growing antral follicle was no longer observed. Emergence of a follicular wave was defined as the day on which the retrospectively traced dominant follicle (DF) was 6 mm while deviation or selection as the day the DF continued growth on expense of the subordinate follicle and the difference between the two follicles reach its maximum (Pastorello et al. 2022). The DF is the first follicle to reach 30mm while the subordinate follicle is the second largest one (Ginther et al. 2004). Number of ovulations, size of the ovulatory follicle, interval to emergence of a new wave, number and size of the CL and growth and regression rates of the follicles and CL were determined and compared between groups. Pregnancy diagnosis was performed by ultrasonography 15 days after AI. The conception rate was determined and compared between groups.

Hormonal analysis

Blood samples were collected during 5 landmarks of each protocol (at the beginning, injection of PGF2α, injection of ovulation inducer, insemination and ovulation) by jugular venipuncture into plain tubes. After centrifugation, the serum samples were kept at -20□C until assay. Concentrations of estradiol-17β (E2) and progesterone (P4) were determined using ELISA kits (Immunocentrix, 22222 Sherman Way, Ste 103, Canoga Park, CA 91303 USA). Variation coefficients for intra- and inter-assay and sensitivity tests were 15.7%, 8.5%, and 0.1ng/ml for P4 and 9.1%, 13.8%, and 10pg/ml for E2, respectively.

Statistical analysis

The data were presented in mean±SEM and statistical analysis was carried out using SPSS program, version 25.0 (2017). T-test was used to compare groups for ovarian findings, hormonal profiles and the interval from treatment to ovulation and to wave emergence. Non-parametric data like ovulation and conception rates were compared between groups by $\chi 2$-test. Level of significance was set at $P \leq 0.05$.

RESULTS

Ovarian findings

At the preliminary period prior to application of the synchronization protocols, mares showed normal estrus behavior at teasing (accepting the approach of the teaser, leans toward the teaser, frequent urination and winking) for average duration of 4.55±0.27 (3-9 days). The cyclic CL lasts for 15.23±2.45 days and the average estrus cycle length was 21.84±5.18 days. Table 1 summarizes the ovarian findings during the ovulation synchronization protocols compared to the G-P-PG-H(D19) group. The intervals to the follicular emergence (P=0.02) and deviation (P=0.04) were shorter and the follicular growth rate was slower (P=0.01) in GPG than G-P-PG-H(D19) group. Mares treated with G-P-PG-H(D19) protocol had a slower CL regression rate than mares in the GPG group (P=0.04). All mares ovulated in response to both GPG and G-P-PG-H(D19) protocols. Type and day of synchronization protocols had no significant effect on the follicular population and follicular waves.

Right ovulation was non-significantly more frequent (54.2%) than left ovulation (45.8%) Relative to the previously-detected CL, 40% of the DF emerged and ovulated from the ipsilateral ovaries, compared to 50% from the contralateral ovaries.

Three mares ovulated 48 hrs (24 hrs after AI) and 2 mares ovulated over 120 hrs in the G-PG-G(D9) subgroup. Ovulations took place within 120-192hrs after the second GnRH in all 5 mares of the G-PG-G(D12) subgroup. In the G-P-PG-H(D19) group, 8 mares ovulated within 36hrs following the administration of hCG (12 hrs after AI) while the remaining 2 mares ovulated over 60hrs.

GPG-treated mares had a longer estrus duration (P=0.02), larger DF (P=0.01), longer GnRH-ovulation and AI-ovulation intervals (P=0.003), compared with G-P-PG-H(D19)-treated mares (Table 1). By Day 9 in both G-PG-G(D9) and G-PG-G(D12) subgroups, only 4/10 mares had follicles over 30mm emerged following the first GnRH. Number of ovulated follicles did not differ between groups. Conception rates (CR) were 0/5 (0%), 1/5 (20%) and 5/10 (50%) for the G-PG-G(D9), G-PG-G(D12) and G-P-PG-H(D19) treated mares (Table 2). Out of the 6 conceived mares, 5 mares had right ovulation. Out of the 5 conceived mares in G-P-PG-H(D19) group, 4 mares had right ovulation and ovulated within 36 hrs of the hCG injection (12 hrs after insemination). In non-conceived mares in all treated mares, 64.3, 63.6 and 57.14% had left ovulations. Twin pregnancy was found in one mare in the G-P-PG-H(D19) group.

Hormones

Fig. 2 and 3 showed the changes in both E2 and P4 during the course of the synchronization protocols. In GPG treated mares, E2 did not change significantly among days of treatment (P=0.9) while P4 changed significantly among days of treatment (P=0.0001), decreased sharply after PG treatment in both G-PG-G(D9) and G-PG-G(D12) subgroups. In G-P-PG-H(D19)-treated group, both hormones did not change significantly among treatment days. In general, P4 in the studied mares showed a marked decrease following the end of the synchronization protocols after induction of ovulation and continued its decrease throughout days of insemination and ovulation.

Table 1: Ovarian response of Arabian mares to GPG and G-P-PG-H(D19)–synchronization protocols

Ovarian response to the programs' cascades		GPG		G-P-PG-H(D19)
		G-PG-G(D9)	G-PG-G(D12)	
Ovarian findings at D0	CL (n)	1/5	2/5	6/10
	F ≥30mm (n)	3/5	2/5	3/10
Response to first dosage		GnRH	GnRH	Progesterone
Ovulation (n)		0/5	2/5	2/10
Double ovulations		0/5	0/5	0/10
Time to ovulation (h)		-	48±0.02	36.48±1.03
Dominant follicle size (mm)		-	41.5±2.1	43.0±1.06
Follicular waves (n)		3/5	2/5	3/10
Follicular growth rate (mm/day-1)		1.13±1.2[a]	0.78±0.8[a]	2.18±0.79[b]
Ovarian findings at PGF2α		D7	D7	D9
Follicle ≥30mm (n)		2/5	1/5	1/10
CL (n)		0/5	3/5	8/10
Response to PGF2α				
Time to follicular emergence (h)		36.00±17[a]	36.00±17[a]	56.00±1.11[b]
Interval to deviation (hrs)		103.8±8.4[a]	129.6±20.94[a]	88.2±10.76[b]
Follicle ≥30mm (n)		2/5	2/5	0/10
Follicular growth rate (mm/day-1)		0.7±0.9[a]	1.92±0.5[a]	2.31±0.98[b]
Luteolysis (n)		0/5	3/5	4/10
Luteal regression rate (mm/day-1)		-	7.67±7.3[a]	2.66±1.08[b]
Response to third injections		GnRH	GnRH	hCG
No. of DF ≥30mm at the time of injection		3/5	2/5	8/10
No. of ovulated mares		5/5	5/5	10/10

Values (mean±SE) with different letters in a row differ significantly (P=0.05).

Table 2: Effect the synchronized protocol on the estrus, ovulation, and conception rate of Arab mares

Clinical findings	Protocols		
	G-PG-G(D9, n=5)	G-PG-G(D12, n=5)	G-P-PG-H(D19, n=10)
Estrus	7.6±0.39[a]	7±0.71[a]	5.3±0.32[b]
Diameter of the ovulatory follicle	44.4±1.92[a]	40.6±2.65[a]	36.9±0.85[b]
Number of ovulations	1	1.6±0.32	1.4±0.17
GnRH/LH–Ovulation interval (hrs)	88.8±12.25[a]	134.4±17.19[b]	47.4±5.77[c]
AI-Ovulation interval (hrs)	64.8±25.95[a]	110.4±10.33[b]	23.4±7.41[c]
Conception	0/5 (0%)	1/5 (20%)	5/10 (50%)
Right/left ovulation	67.15/42.85	20/80	66.67/33.33

Values (mean±SE) with different letters in a row differ significantly (P=0.05).

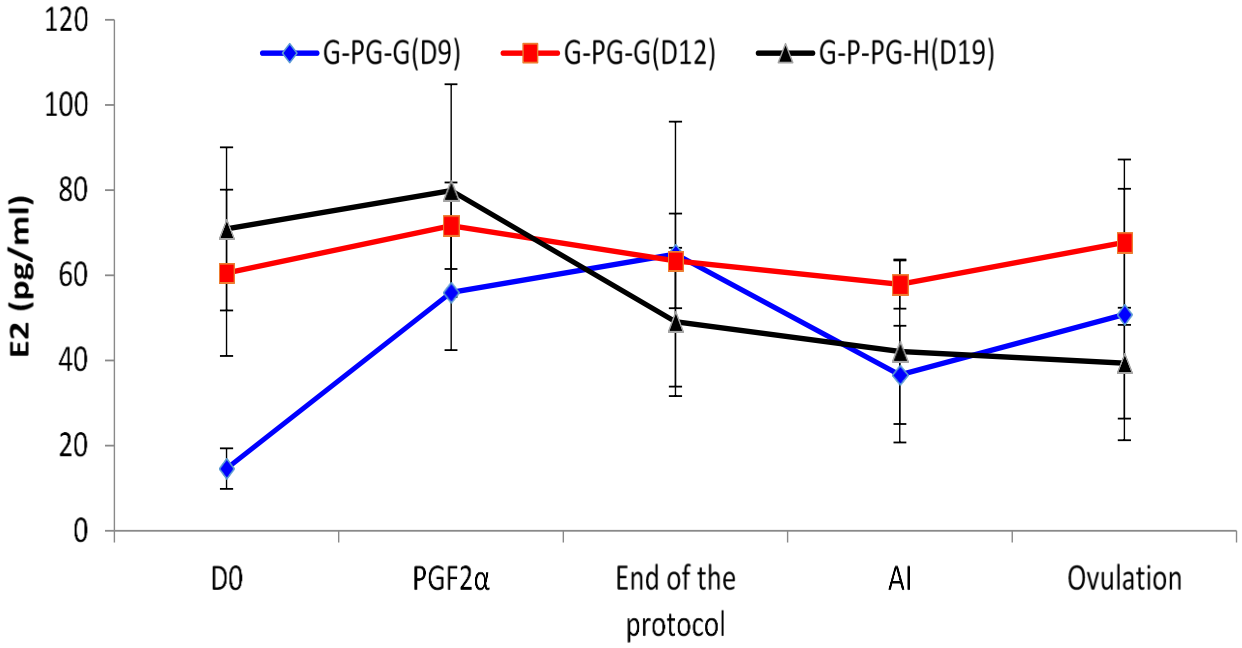

Fig. 2: Average changes in Estadiol-17β (E2) in Arabian mares synchronized for estrus with ovulation synchronization protocols, G-PG-G(D9, n=5; second GnRH on Day 9), G-PG-G(D12, n=5; second GnRH on Day 12) and P-PG-H(D19) (n=10, oral progesterone, PGF injection on D9 of the protocol and hCG on D 19) where D0 is the beginning of the program and the protocols ends with second GnRH for and hCG administration for GPG and P-PG-H(D19)-treated groups, respectively; Days of treatment (D0= the first day of the program).

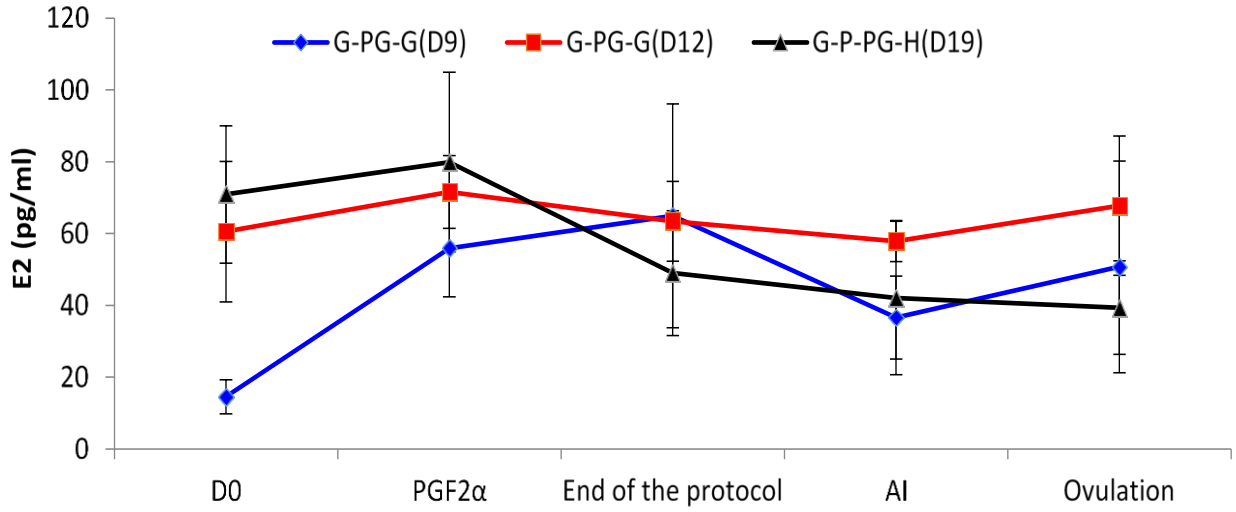

Fig. 3: Average changes in progesterone (P4) in Arabian mares synchronized for estrus with ovulation synchronization protocols, G-PG-G(D9, n=5; second GnRH on Day 9), G-PG-G(D12, n=5; second GnRH on Day 12) and P-PG-H(D19) (n=10, oral progesterone, PGF injection on D9 of the protocol and hCG on D 19) where D0 is the beginning of the program and the protocols ends with second GnRH for and hCG administration for GPG and P-PG-H(D19)-treated groups, respectively; Days of treatment (D0= the first day of the program).

DISCUSSION

The present study demonstrated that the use of GPG protocol in Arabian mares in its present form is unfeasible for promoting reproductive performance regardless the unequivocal ovulation rate. Several factors contributed to this claim including failure to respond satisfactorily to the first dose of GnRH, unharmonized ovulation, the slower growth rate of the DF, the longer second GnRH-ovulation and insemination-ovulation intervals compared with the commonly used progesterone-PGF2α-hCG protocol. There is a general understanding that follicles grow slowly over an extended period are less likely get fertilized due to the production of subfertile oocyte (Cuervo-Arango and Clark, 2010). Similar to the present findings, mean growth rates over ten days prior to ovulation were 2.20mm/day (range 1.18 to 3.64) in draft horse with unsatisfactory pregnancy outcome (Zielinski et al. 2021). Previous reports pointed out to the stage of growth or demise of the detected ovarian structure upon administering hormonal agonists or antagonists is a crucial factor that determines the future fate of anticipated DF (Pastorello et al. 2022). The expected fertility outcome is inversely correlated with the intervals from emergence to selection and from selection to dominance in individual mares (Mesut et al. 2022). It is worth mentioning that postponing the day of second GnRH to Day 12 in this study had no impact either on the ovarian findings or the conception rate. By Day 9 of the GPG protocol, 60% of the mares had DF less than 30mm which considered not ready to respond to the induction of ovulation by second GnRH at least by the due time for insemination and expected fertilization (Pastorello et al. 2022). The other mares having DF ≥30mm (4/10) were originally responding to the first GnRH of the protocol. These mares had minimum P4 concentration compared with peers in the same protocol which may suggest that GPG could be more profitable as long as treated mares were presynchronized to ensure their ovaries contain no or nonfunctional CL.

Timing -not the number or frequency- of inseminations is important for getting mare in foal (Kareskoski et al. 2019). Mares should be aligned to ovulate within 12hrs following artificial insemination using extended semen to optimize conception (Al-Kass and Morrell 2024). Projection onto the present study, it is probably the lack of uniformity of ovulation in the GPG group either relative to second GnRH or –mostly- to insemination is one of the main causes of the failure of conception in this group. As long as the single insemination per cycle is performed between 24hrs and 12hrs before and after AI for cooled semen or between 12hrs before and 12hrs after AI for frozen-thawed semen, it produces pregnancy rates as good as multiple inseminations under conditions of frequent veterinary examination (Sieme et al. 2003). Despite the fact that equine spermatozoa may survive in the female tract for 24-48hrs especially in the uterotubal junction or the oviductal isthmus (Bucca and Carli 2011), their fertilizability decreases after the first 12hrs after ejaculation either due to aging while waiting for the ovulated oocyte or demise of the fertilized ovum (Ginther 2017). In 8 out of 10 G-P-PG-H(D19)-synchronized mare s in the present study, ovulation was detected 36hrs following hCG administration (12hrs after insemination) which may be consistent with the aforementioned quote. Accordingly, it was mentioned that the efficacy of any ovulation-inducing agent is playable by the stage of the cycle, follicular diameter and ripeness (Samper 2008). Most equine practitioners consider ovulation beyond 48 hrs after the administration of the inducing agents is irrelevant and inappropriate (Mesut et al. 2022) which may explain the unsatisfactory outcome of the GPG protocol in this study relative to the inconsistent ovulation time among synchronized mares.

In the present study all mares in different groups were inseminated once 24hrs after the administration of the ovulation inducer (second GnRH or hCG). Number of inseminations has been reported to have a limited effect on the pregnancy outcome in synchronized mares as long as

the mares inseminated in the appropriate time relative to ovulation (Karam et al. 2017). Moreover, factors like breed of the mare, frequency of breeding in the same cycle, number and percentage of progressively motile spermatozoa are detrimental factors for the outcomes of any synchronization program (Derisoud et al. 2022). It was claimed that repeating AI on a daily basis optimizes fertility if frequent scanning is not possible. Even in synchronization protocol, dealing with individual mares according to the size of the ovulatory follicle (≥35mm) upon administration of hCG is more advantageous to the mare's fertility than indiscriminate blind insemination (Shore et al. 1998).

The mean diameter the ovulatory follicle of the GPG groups was greater than that of the G-P-PG-H(D19) group in the present study. However, it is worth mentioning that at the time of administration of second GnRH and hCG, 50% and 80 % of the mares had follicles ≥30mm for the GPG and G-P-PG-H(D19) groups, respectively. There is a general agreement that 30-35mm ovulatory follicles are more likely responsive to ovulation induction in mares (Samper 2008). Moreover, in the GPG treated mares in the present study, 5 mares had 19-28mm follicles at the second GnRH which may explain the longer time needed for these follicles to reach the ovulatory size and the failure of these mares to consistently ovulate in an optimum time for ovulation and/or insemination. Earlier reports pointed out to a critical role of the diameter of the DF on the conception rate in Arabian horse (Elmetwally et al. 2017). In accordance with the findings of the present study, the growth rate of the DF in Arab mares ranged from 2-7mm per day till the last 48hrs before ovulation (Warriach et al. 2014). Prior to impending ovulation of Arabian mares, the DF either remained unchanged or showed a noticeable reduction in size (Karam et al. 2017). More Arabian mares conceive when the diameter of the ovulatory follicles range between 35-44mm than follicles over 44mm (Najjar et al. 2018).

Side of ovulation has been claimed to affect conception in mares (Kareskoski et al. 2019). Right ovulating cycles are more fertile than left ones (Yurdaydin et al. 1993 and Najjar et al. 2018). In the present study, it was interesting to notice that 5/6 of the conceived mares had right ovulations which probably support the above assumption. On the other hand, others cited that these findings irrelative to conception in mares and demonstrated that the activities of the ovaries in mares are nearly equal and tend to be more left than right (Elmetwally et al. 2017). However, the few number of mares used in this study may limit its feasibility and these findings should be carefully considered. In the present study, the overall conception rate of 50% for the G-P-PG-H(D19) group was lower than previous report of 60-70% (Rota et al. 2004) but higher than the rate in a group of Arabian bred mares (37%) hormonally treated with hCG (Najjar et al. 2018; Derisoud et al. 2022).

Owing to the presence of mature CL in nearly 50% of the studied mares in different groups at the beginning of each protocol, there was a notable high level of progesterone in the first 2 samples of the present study which readily decreased following the administration of PGF2α by Day 7 in the GPG group and Day 9 in the G-P-PG-H(D19) group. These results are consistent with previous findings of Pastorello et al. (2022). Concerning the insignificant changes in E2 are probably due to the relatively long intervals between samples taken to trace its profile in the present study. E2 is subjected to dramatic fluctuations around the period of ovulation in normally cyclic mares and plateaued 2 days prior to ovulation which necessitate the daily sampling during this period to track these changes (Cuervo-Arango and Clark 2010; Zielinski et al. 2021).

It can be concluded that failure to respond satisfactorily to the initial dose of GnRH, failure of some ovulatory follicles to reach the appropriate size by the predesigned time for the second GnRH, slow growth rate of the ovulatory follicle, long GnRH-ovulation and AI-ovulation intervals are probably factors involved in the unfeasibility of the Ovsynch protocol in Arabian mares in the present study. Accordingly, it is recommended to modify this protocol in regard to the timing of the administered hormones or the insemination time to increase its profitability in equine reproduction. Also, it can be suggested that mares can be presynchronized with 14-days double PGF2α injections before the Ovsynch protocol and mares closely scanned at impending ovulation and inseminated consequently.

Author Contribution Statement

Derar Refaat: Conceptualization, Methodology, Writing- Original draft preparation, Reviewing and Editing; Ahmed Ali: Conceptualization, Methodology, Data curation, Writing- Reviewing and Editing; Anas Aljibali: Clinical examination, Data collection; Mohamed Ali: Hormonal analysis, Consultation.

Conflict of interest

None

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Data availability

Data is available upon reasonable request from the authors of the manuscript

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