



Impact of Various Exogenous Gonadotropins and Their Combination on the Enhancement of Conception Rate in Equines

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ABSTRACT

Background: Mares are seasonal breeders, active in spring and summer when daylight is longer, ensuring foals are born in spring for ideal conditions. Having more foals in a breeding season is desirable for stud owners to improve their economic returns.

Methods: Hormonal treatments with hCG and GnRH analogs can enhance fertility of mares inducing ovulation in follicles measuring over 35 mm in diameter, helping to synchronize and improve breeding. In the present study 100 indigenous mare breeds were selected from Khyber Pukhtunkhwa and Punjab province during February to October 2024-time period. They were divided in to 4 experimental groups based upon the hormone treatment given i.e. GnRH, HCG, GnRH and HCG combination and control groups.

Result: The pregnancy rate owing to treatments used was significantly higher than the control group ($p \leq 0.05$), as determined by Chi-square analysis. The pregnancy rates were 60%, 68%, 72% and 32% for GnRH, HCG, GnRH and HCG and control groups respectively. No significant difference was observed between the first three treatment groups. Uterine edema was analyzed for its influence on pregnancy outcomes. The analysis using Spearman's correlation revealed a correlation coefficient of -0.043 with a p-value of 0.691. This indicates statistically non-significant relationship between the degree of uterine edema and pregnancy outcomes between the treatment groups. These findings suggest that hormonal treatments may offer a promising approach to enhance fertility and conception rates in mares, particularly in indigenous breeds of Pakistan.

Key words: Breeding, Economic empowerment, Equine, Exogenous hormones, Reproductive.

INTRODUCTION

Equines are seasonal breeders and their reproductive cycle is influenced by duration of day light, particularly in temperate areas (Pal *et al.*, 2021; Khosa *et al.*, 2023). Mares' reproductive cycle is active during spring and summer seasons when the day length is long while during winter and autumn seasons they remain anestrus owing to the shorter days and duration of day light (Aurich, 2011; Cortés-Vidauri *et al.*, 2018). This behavior allows foals born during spring when the environmental conditions are more suitable (Guay, 1980). For stud owners, having more foals each year is important for both financial success and the ongoing development of high-quality horses (Ogbanya *et al.*, 2020). By breeding more foals, owners can choose the best horses for racing, competition and breeding, helping to improve the breed. This also meets market demand and gives owners a chance to showcase horses with the potential to succeed, whether in racing or shows (Hallman *et al.*, 2023).

Proper use of gonadotropins could enhance fertility and improve conception rate and eventually could improve the number of foals born per breeding season. The hormones commonly used are human chorionic gonadotropin (hCG) and gonadotropin-releasing hormone (GnRH) analogs. hCG acts as luteinizing hormone which induce ovulation in 36-48 hours when the follicle diameter is greater than 35 mm (Alyasin *et al.*, 2016). Similarly, GnRH

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analogues induce the release of endogenous LH and FSH from anterior pituitary gland and induce ovulation when follicle diameter is greater than 35 mm (Hassanein *et al.*, 2024). These treatments help in timed ovulation with artificial insemination or natural mating, improving breeding success. Combination therapy using both GnRH and hCG were used to get synergistic response in inducing ovulation

(Zhou *et al.*, 2022). However, research indicated that combination did not significantly improve the ovulation and conception rate compared to single hormone treatment protocols (McCue *et al.*, 2007).

Several studies have questioned the effectiveness of using hCG, GnRH and their combination in improving conception rates during the breeding season (Tazawa *et al.*, 2017; Fanelli *et al.*, 2022), indicating the need for further research to fully understand their potential benefits. However, there is limited data on how indigenous horse breeds in Pakistan respond to these hormonal protocols. Therefore, the present study was designed to evaluate the efficacy of GnRH, hCG and their combination in enhancing ovulation and conception rates specifically in Pakistani indigenous mares during the breeding season.

MATERIALS AND METHODS

Selection of animals

The experimental study was performed on 100 horses randomly selected from different districts of KPK and Punjab, Pakistan. All the horses were native breeds or non-descript breeds between the age group of 5-12 years with body condition score above 4. All the mares were healthy with normal estrus cycle and no disease in the uterus. The data were collected between February and October 2024, during which the average environmental temperature was $27.67^{\circ}\text{C} \pm 5.74^{\circ}\text{C}$ and the relative humidity was $63.33\% \pm 1.56\%$.

Treatment groups

The study was conducted on field mares reared as pets, where each mare or a small group of mares was kept under semi-intensive management conditions. One hundred mares were randomly divided in to 4 groups; GnRH group (n=25), hCG group (n=25), GnRH and hCG group (n=25) and control group (n=25). All the mares were checked with ultrasound machine to determine the size of developing follicle. The treatment was initiated until the follicle size diameter was greater than 35 mm. In GnRH group, a GnRH analogue leirelin acetate 100µg (Inj Dalmeralin 4 ml, Fatro, Italy) was injected IM 8 hours before covering. In hCG group, hCG 1500 IU (Pregnyl, Organon, US) was injected IM 8

hours before covering. In GnRH and hCG group, both GnRH 100 µg and hCG 1500 IU was injected IM 8 hours before covering. In control group, normal saline 2 ml was injected 8 hours before covering. The mares were checked for pregnancy via ultrasonography 20 to 30 days after the last covering.

Before covering, all mares were sonographically examined for the presence of uterine edema using the subjective scoring system (0-5) as previously described (Samper, 1997). Mares in diestrus with no visible uterine edema were assigned a score of 0, while those with the highest level of edema received a score of 5. Scores from 1 to 4 were given based on the increasing severity of edema observed (Samper, 1997).

Pregnancy rates among the treatment groups were analyzed for statistical significance using the Chi-square test. The data were segregated and summarized using means and standard deviations. The relationship between uterine edema and pregnancy outcomes was assessed using Pearson's correlation analysis in IBM SPSS Statistics (Version 29.0), without considering the effects of hormonal treatment or treatment group.

RESULTS AND DISCUSSION

The conception rate was higher in the treatment groups as compared to control group. No significant difference was observed among the three hormonal groups. The pregnancy rate was 60%, 68%, 72% and 32% for GnRH, hCG, GnRH and hCG and control groups respectively (Table 1). The relationship between uterine edema and pregnancy outcomes is often considered an important factor in reproductive studies, with the expectation that optimal edema levels may positively influence conception (Grabowska and Kozdrocki, 2022). However, in the present study, Spearman correlation analysis revealed a negative correlation between uterine edema grades and pregnancy outcomes ($r = -0.043$), with a p-value of 0.691. This result indicates no statistically significant association, suggesting that variations in uterine edema scores did not meaningfully affect the likelihood of pregnancy (Table 2).

For mares in a seasonally anestrous, GnRH or its analogues stimulate the anterior pituitary gland to release

Table 1: Pregnancy outcomes in different treatment groups.

Groups	Pregnant	Non-pregnant	Total	Pregnancy (%)
GnRH group	15	10	25	60
HCG group	17	8	25	68
GnRH and HCG	16	9	25	64
Control	8	17	25	32

Table 2: Pregnancy out comes with respect to different edema levels.

Groups	Edema level 1	Edema level 2	Edema level 3	Edema level 4
GnRH group	5	6	8	6
HCG group	5	7	9	4
GnRH and HCG	7	6	8	4
Control	5	6	6	8

LH and FSH, which causes ovulation (Hassanein *et al.*, 2024). Based on available information, follicle size at treatment time, anestrus stage and the method of administration all seem to have an impact on the effectiveness of GnRH therapy (Mu *et al.*, 2019). Compared to mares with follicles less than 15 mm, transitional mares with follicles larger than 25 mm in diameter are often more likely to react (Papas *et al.*, 2021). Using 40 µg of buserelin, the ovulation rate was 100% in heavy draft mares with follicles measuring approximately 45 mm (Miki *et al.*, 2016). Ovulation in the early and late fall was successfully induced in 90% of mares by deslorelin acetate (Spencer *et al.*, 2022). Our results agree with earlier data. The current study's results indicate that GnRH is beneficial in increasing equines' fertility rate because the GnRH group's pregnancy rate was higher than that of the control group.

Administering 1500 IU of human chorionic gonadotropin (hCG) intravenously to mares with follicle diameters of 35 mm or greater led to enhanced ovulation and conception rates. Additionally, it led to enhanced growth of the early conceptus and elevated progesterone levels in the circulation (Aurich *et al.*, 2019). Elevated doses of hCG, namely 3000 IU IV (Morel and Newcombe, 2008) or 2500 IU IV (Boakari *et al.*, 2017), led to enhanced follicular maturation and reduced endometrial folding. To economize and inhibit the formation of hCG antibodies, a reduced dosage of hCG (450 IU) was administered intravenously at the Hou Hai acupoint, consequently inducing ovulation in donkeys (Ribeiro *et al.*, 2020). The evaluation of several hCG dosages, specifically 1500 IU and 3000 IU (GnRH) in mares, revealed that 1500 IU was more effective in inducing ovulation within 48 hours, achieving a peak pregnancy rate of 93.33% (Phetudomsinsuk, 2017). The efficacy of hCG in stimulating ovulation is dependent upon the follicle's size. As the follicle enlarges to a diameter of 35 mm, its sensitivity to hCG intensifies (Fanelli *et al.*, 2022). The current study demonstrated a greater pregnancy rate in the hCG group compared to the control group, consistent with other research findings.

The degree of uterine edema has been suggested by some authors as a predictor of improved pregnancy rates. For instance, uterine edema was found to be positively associated with higher pregnancy rates in Lusitano mares (Mata *et al.*, 2013). Additionally, Mateu-Sánchez *et al.* (2016) observed that uterine edema increased with the duration of estrus in both spontaneous and PGF-induced estrus cycles, leading to higher pregnancy rates. However, other studies suggest that while uterine edema may help determine the optimal time for insemination, it is not significantly correlated with improved pregnancy outcomes. For example, Tirpan *et al.* (2024) reported no significant differences in uterine edema values between pregnant and non-pregnant mares across two consecutive ovulations with respect to pregnancy outcomes. Results of the present study align with those of Tirpan *et al.* (2024), where no significant relationship between uterine edema and pregnancy was observed.

Research comparing various GnRH analogs with hCG has shown no significant differences in effectiveness among buserelin, deslorelin (Ferreira-Silva *et al.*, 2018), histrelin (Voge *et al.*, 2012), lecorelin (Vavasseur *et al.*, 2023) and triptorelin (Dordas Perpinyà *et al.*, 2020). Deslorelin in a liquid formulation has been found to be as effective as hCG and pelleted deslorelin in inducing ovulation (Blanchar *et al.*, 2002). Additionally, the response to hCG and histrelin acetate appears to vary with the age of the mare. For mares under 8 years of age, both hCG (1500 IU IV) and histrelin acetate (250 µg IM) were equally effective, while mares over 15 years of age exhibited greater sensitivity to histrelin for ovulation induction during the breeding season (Cazales *et al.*, 2023). In the present study, hCG demonstrated similar efficacy to GnRH analogs but was significantly more effective than the control group (Table 1).

Equine practitioners often recommend using a combination of hCG and GnRH to induce ovulation, citing potential synergistic effects. However, studies have shown no such synergistic advantage. For instance, the group receiving both a GnRH analog and hCG demonstrated similar ovulation timing compared to groups treated with hCG, deslorelin, or histrelin alone (Chopin *et al.*, 2020). In embryo recipient mares, this combination had no impact on corpus luteum formation, progesterone levels, or fertility rates (Segabinazzi *et al.*, 2021). Many studies have evaluated the influence of hCG and GnRH on fertility and conception rates during the breeding season, with many findings indicated no significant enhancement in the desired outcomes. Similarly, result of the present study revealed no synergistic benefit from combining hCG and GnRH compared to using either hormone alone. However, the pregnancy rate in the combination group was significantly higher than in the untreated control group (Table 1).

Many studies have reported findings that contradict the potential benefits of gonadotropins in enhancing pregnancy rates. The administration of hCG did not result in a significant improvement in pregnancy rates compared to control groups (Johnson and Martin, 2004; Brück and Hecker, 2010). Similarly, while GnRH analogs were effective in inducing ovulation, they did not led to a notable increase in conception or pregnancy rates (Carnevale and Ginther, 1995; Ball and Little, 1991). Result of the present investigation revealed significantly higher conception rates in the treatment groups compared to the control group, highlighting their potential role in enhancing reproductive success.

CONCLUSION

Pakistan's indigenous thoroughbred horses are kept as pets, played in traditional games, and exhibited at various festivals. Proper breeding and the production of numerous foals in a single season have the potential to improve the financial well-being of breeders. The use of GnRH or HCG may help increase conception rates in Pakistani indigenous thoroughbred horses.

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Conflict of interest

The authors declare that they have no competing interests.

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