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Factors Affecting the Reproductive Efficiency of American Mammoth Jackstock Donkey Mares (*Equus asinus*) under Subtropical Conditions of Pakistan

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ABSTRACT

Donkeys are an integral part of the rural culture of Pakistan, with American Mammoth donkey stallions particularly valued for mule production. This study investigated factors influencing reproductive performance in donkey mares (jennies), focusing on hormonal treatments for estrus/ovulation induction and stallion selection on pregnancy outcomes. The study was conducted in Sargodha, Pakistan (32.0740° N, 72.6861° E) and divided into two parts. The first was a five-year retrospective analysis of breeding and foaling patterns in 321 jennies under subtropical conditions. The second part was a prospective trial involving 60 jennies randomly assigned to three groups: GnRH, hCG, and control (n=20 per group). For estrus induction, 28 jennies (n_1=28) with a corpus luteum received prostaglandin (PG), while 32 jennies (n_2=32) exhibited natural estrus. All were bred using three randomly assigned American Mammoth donkey stallions. Chi-square tests evaluated the effects of estrus type and ovulatory treatments on ovulation rate. Binary logistic regression assessed the impact of estrus type, ovulation induction, post-breeding induced endometritis (PBIE), rebreeding, and stallion used on pregnancy rate. One-way ANOVA analyzed seasonal and monthly breeding and foaling patterns. Results showed a pregnancy rate of 68.33% (41/60). GnRH (75%) and hCG (80%) groups had higher pregnancy rates than the control (50%) (*P* = 0.048). Ovulation rates were 100% in GnRH and hCG groups versus 85% in controls (*P* = 0.043). Mean follicular sizes were not significantly different among groups (*P* = 0.108). PG-induced estrus resulted in a 57.1% pregnancy rate compared to 78.1% in natural estrus jennies, though not statistically significant (*P* = 0.307). PBIE occurred in 13.3% (8/60), but oxytocin treatment showed no significant impact (*P* = 0.486) on pregnancy rate. Rebreeding yielded 57.1% success versus 80.5% in single breeding (*P* = 0.307). Stallion choice significantly affected pregnancy outcomes (*P* = 0.024). Seasonal and monthly variations in breeding/foaling rates were non-significant (*P* > 0.05). In conclusion, American Mammoth jennies under subtropical conditions do not exhibit strict seasonality. Ovulation induction (GnRH, hCG) and stallion selection significantly influence pregnancy success, providing key strategies for optimizing donkey reproduction in Pakistan.

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Key words
Donkey mares, Estrus induction, GnRH, hCG, Oxytocin

INTRODUCTION

Donkeys (*Equus asinus*) play an integral role in Pakistan's rural economy, particularly in transportation and agriculture. With a national population of approximately 5.9 million (Pakistan Economic Survey, 2023–24), these animals contribute significantly to labor-intensive tasks where mechanization is impractical (Khan *et al*., 2020). Among the various breeds, American Mammoth Jackstock stallions are highly valued in Pakistan for their role in mule production due to their size and strength. In subtropical regions like Pakistan, donkeys significantly contribute to

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economic stability by performing labour-intensive tasks unsuitable for mechanization (Earnist *et al*., 2022; Khan *et al*., 2022). Globally, the focus on donkey reproduction is growing due to their utility and economic importance (Miragaya *et al*., 2018).

Unlike horses, donkeys display a more flexible seasonality in their reproductive cycles, allowing for potential year-round breeding. However, the reproductive efficiency of donkeys is influenced by factors such as photoperiod, ambient temperature, and nutrition, necessitating a well-managed breeding program to optimize fertility and successful outcomes (Canisso *et al*., 2019). Developments in reproductive biotechnologies like artificial insemination and embryo transfer are crucial for conserving genetic diversity and improving reproductive efficiency, offering significant benefits for breed conservation and sustainable livestock management (Gambini *et al*., 2025).

The strategic use of hormones such as gonadotropin hormone-releasing hormone (GnRH), human chorionic gonadotropin (hCG), and prostaglandin (PG) is critical in managing fertility in donkeys. These hormones induce ovulation and manage estrous cycles, enhancing reproductive management. GnRH and hCG are used for inducing timely ovulation essential for synchronized breeding, while PGs are utilized for their luteolytic effects, aiding in estrus synchronization (De Oliveira *et al*., 2019; Horáčková *et al*., 2019; Mahmood *et al*., 2024; Oliveira *et al*., 2020).

Ovulation and pregnancy rates in donkey mares and other equine species are affected by various factors, including the animal's age, health status, environmental conditions, management practices, and nutrition. Stress from poor health or suboptimal living conditions can also disrupt the reproductive cycle (Mahmood *et al*., 2024).

Previous studies have reported a significant effects of stallion factors on pregnancy rate in jennies. The stallion's genetic quality, health, and fertility influence breeding outcomes. Proper stallion management, including health checks and optimal nutrition, is crucial for maintaining fertility and achieving high pregnancy rates in breeding programs (Aıssanou *et al*., 2022; Rota *et al*., 2018; Zakari *et al*., 2015).

The reproductive efficiency of donkey mares is affected by various reproductive disorders, including donkey mares (Wang *et al*., 2024). Post breeding induced endometritis (PBIE) is a significant reproductive disorder affecting the fertility of donkey mares. Managing this condition is crucial as it involves inflammation of the endometrial lining following breeding, which can hinder successful implantation and pregnancy. Techniques such as timely use of oxytocin and monitoring through ultrasonography are essential for managing this condition effectively, thereby minimizing its impact on fertility and enhancing overall reproductive success (Karam *et al*., 2021; Khan *et al*., 2025). Effective reproductive management of donkeys is vital for sustaining their population and ensuring their continued contribution to local economies (Khan *et al*., 2025).

Despite growing global interest in donkey reproduction, few studies have explored these variables under subtropical field conditions, especially in the American Mammoth Jackstock breed. This presents a significant research gap. Therefore, the present study was designed to investigate the seasonal breeding patterns and the effects of ovulatory treatments (GnRH, hCG), PG-induced estrus, PBIE, rebreeding, and stallion selection on pregnancy outcomes in American Mammoth Jackstock donkey mares under the subtropical conditions of Pakistan.

MATERIALS AND METHODS

Study location and animals

The study was conducted at Donkey Breeding Stud, located in Sargodha district (32.0740° N, 72.6861° E) of Punjab, Pakistan. The study involved American Mammoth Jackstock donkeys, originally imported from the USA, but all individuals used were locally born in Pakistan. For the study about retrospective analysis of the breeding pattern of donkey mares (jennies), the breeding record of three hundred and twenty-one jennies was evaluated. For the study about the factors affecting the fertility outcomes in the donkey mares, sixty jennies and three donkey stallions were selected. All the jennies and stallions involved in the second part of the study were screened for breeding soundness (McCue, 2021) and having 5-8 years of age.

Animals were housed in well-ventilated shelters, with a space allowance of 3.5–4 m² per animal. The flooring consisted of non-slippery surfaces and straw or rubber bedding. Donkey mares were kept in groups of ten per paddock, while each stallion had a separate stall. Fresh water was available *ad libitum*. The diet included lucerne (alfalfa) and crushed grains (barley and gram), provided according to established feeding standards (Martin-Rosset, 2018).

Study design

The study was conducted to investigate seasonality patterns and factors affecting the reproductive performance of donkey mares in Pakistan. There were two parts of the study; in the first part, a retrospective analysis of the breeding pattern of donkey mares over the last five years (2019-2023) was performed. This part evaluated the effect of different seasons of the year on breeding and foaling patterns. In total there were 321 breedings and 112 foalings recorded during these five years. The effect of the season and month of the year on the frequency of breeding and foaling in the donkey mares.

In the second part of the study, sixty donkey mares were selected, and the effect of the ovulatory treatment (GnRH vs hCG) and control group was divided. The donkey mares were randomly divided into three treatment groups, i.e., GnRH group (n_1 = 20), hCG group (n_2= 20), and control group (n_3=20). The selected mares were initially scanned through ultrasonography for the presence and absence of corpus luteum (CL). Three donkey breeding stallions were selected for breeding the donkey mares based on the breeding soundness exam and previous breeding results. The donkey mares having CL were treated with PG. There were only twenty-eight mares which were having CL and were treated with PG to induce estrus. The mares in estrus were followed through ultrasonography every 12 h till one day post-ovulation. The donkey mares of treatment groups (once in estrus) were treated with ovulatory treatment once the size of the dominant follicle crossed 35 mm and the uterine edema score dropped to 1. The control group of mares was followed similarly but received normal saline injection only instead of GnRH or hCG. The donkey mares were bred naturally one day post ovulation induction treatment, and rebreeding was also an option if donkey mares failed to ovulate within 48 h after first breeding. The donkey mares were treated with oxytocin hormone if PBIE was observed. The pregnancy was checked on day 14, day 30, and day 60 post-ovulation through ultrasonography. The effect of the independent variables on dependent variables was determined through statistical analysis.

Hormonal treatments

PG was used for luteolysis in the CL having donkey mares. PG used in the study was damazine (manufactured by Fatro S.p.A, Itlay), which is a synthetic analog of PGF2α (0.075mg/ml of cloprostenol). 2ml of dalmazine was given to 28 donkey mares for luteolysis/estrus induction, while 32 mares exhibited natural heat.

For GnRH, dalmarelin (manufactured by Fatro S.p.A, Itlay) containing Lecirelin acetate, which is a long-acting synthetic analogue of hypothalamic GnRH, was used. The dose of dalmarelin was 2ml which was administered intravenously to 20 donkey mares for ovulation inducting by stimulating the release of gonadotropins (FSH and LH) from the pituitary gland.

For hCG, IVF-C injection (Galaxy Pharma (Pvt.) Ltd) was used. This hormone was administered at the rate of 2500 IU per donkey mare, intravenously for inducing ovulation. Oxytocin (Venus Pharma) was used in mares suffering from post-breeding induced endometritis (PBIE). Only eight donkey mares exhibited PBIE and were treated with 2ml oxytocin just after ovulation.

Breeding method

Mares in estrus were bred via natural service using one of the three stallions. If ovulation did not occur within 48 h of the initial mating, rebreeding was performed. Only 14 mares were rebred, and the numbers of donkey mares bred with each stallion were twenty-three for stallion-1, nineteen for stallion 2, and eighteen for stallion-3.

Ultrasound methodology

For ultrasonography, an HS-1600 ultrasound machine (Honda Electronics) equipped with a 7.5 MHz linear rectal probe was used. Initially, a baseline ultrasound examination was conducted on all donkey mares prior to PG administration to evaluate the presence of a CL, the size of the dominant follicle, and the condition of the uterus. Following PG treatment, subsequent scans began on day 5 to monitor luteolysis response and changes in follicular dynamics and uterine edema scores. Mares in estrus were scanned every 12 h until one day post-ovulation. Pregnancy detection was performed on days 14, 30, and 60 post-ovulation.

Data collection for retrospective analysis of breeding patterns in donkey mares

The data of three hundred and twenty-one breedings and one hundred and twelve foalings recorded during these five years was collected and analyzed. The effect of the season of the year and month of the year on the frequency of breeding and foaling in the donkey mares was determined.

Study variables

For the retrospective analysis study, the season and month of the year were independent variables, and number of breedings and foalings were dependent variables. For the clinical trial of ovulation induction and pregnancy in donkey mares, the dependent variables were ovulation rate and pregnancy rate, and the independent variables were PG-treatment, ovulatory treatment PBIE, rebreeding, and stallion used for breeding.

Statistical analysis

All the data were analyzed using SPSS version 27.0, and all statistical tests were performed at a 95% confidence interval. Descriptive data are presented as mean ± standard error of the mean (SEM), while inferential results are reported with corresponding test statistics and P-values. The normality of the data was checked using the Shapiro-Wilk test. The effect of PG treatment and ovulatory treatment on ovulation rate was analyzed using the Chi-square test. The effects of PG treatment, ovulatory treatment, PBIE, rebreeding, and stallion used for breeding on pregnancy rate were analyzed through binary logistic regression. Model fitting for the binary logistic regression was evaluated using the Omnibus test of model coefficients and the Hosmer-Lemeshow goodness of fit test. The effects of season and month of the year on breeding and foaling frequency were analyzed using one-way ANOVA.

RESULTS

Effect of season on breeding and foaling pattern

There was no statistically significant difference in breeding and foaling frequencies of donkey mares among seasons under the subtropical conditions of Pakistan ($P > 0.05$). The results are presented in Figure 1. Analysis of seasonal breeding trends revealed minor numerical variation. The average number of monthly breedings was 5.20 ± 1.38 in spring, 5.47 ± 0.61 in summer, 6.60 ± 1.63 in fall, and 4.80 ± 1.02 in winter. The overall annual mean was 5.35 ± 0.47. Despite these seasonal fluctuations, the Test of Homogeneity of Variances (Levene's statistic = 0.064; df = 3, 56; P = 0.979) indicated that the variance in breeding frequency remained consistent across seasons. Furthermore, one-way ANOVA revealed no statistically significant difference in the mean number of breedings among seasons (F(3, 59) = 0.317; P = 0.813), suggesting that seasonal changes did not have a measurable effect on breeding frequency.

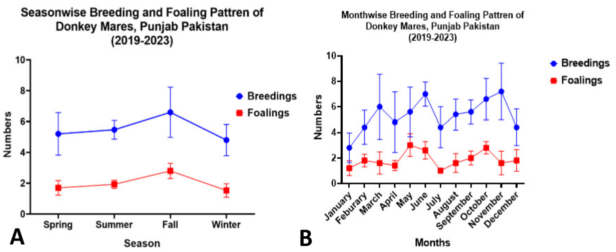

Fig. 1. Seasonal (A) and monthly (B) variation in breeding and foaling frequencies (mean±SEM) of donkey mares under subtropical conditions of Punjab, Pakistan (2019-2023).

Effect of months on breeding

The analysis of monthly breeding services revealed a fluctuating pattern throughout the year, as illustrated in Figure 1. However, the differences in the mean number of services per month were not statistically significant (P = 0.849). This indicates that the observed variability was likely due to random fluctuations rather than systematic monthly differences.

Effect of season on foaling

Seasonal patterns in foaling activity showed numerical variation among donkey mares, as illustrated in Figure 1. The mean number of monthly foalings was 1.70 ± 0.47 in spring, rising slightly to 1.93 ± 0.26 in summer. It peaked in fall at 2.80 ± 0.49 and declined to 1.53 ± 0.44 in winter. The overall seasonal average was 1.87 ± 0.19. Despite these fluctuations, Levene's test indicated no significant difference in variance among seasons (Levene statistic= 0.547, df= 3, 56; P = 0.652), suggesting consistent dispersion in foaling frequency throughout the year.

Effect of months on foaling

Monthly variation in foaling frequency was observed throughout the year. The mean number of foalings ranged from 1.00 ± 0.00 in July to a peak of 3.00 ± 0.89 in May. The overall monthly average for the year was 1.87 ± 0.19. These values, along with their respective standard errors and confidence intervals, provided insight into the precision of monthly estimates. The pattern is further illustrated in Figure 1.

The monthly foaling range extended from a minimum of 0 (recorded in several months) to a maximum of 6 (in May). However, one-way ANOVA revealed no statistically significant difference in foaling frequency across months (F(11, 48) = 0.868; P = 0.576), suggesting that the observed variation was likely due to random fluctuations rather than any systematic monthly trend.

Effect of various factors on pregnancy in donkey mares

The overall pregnancy rate in this trial was 68.33% (41/60). Binary logistic regression analysis revealed statistically significant predictive value (Omnibus test of model coefficients: $\chi^2(7) = 15.532$, P = 0.030). The model effectively differentiated between pregnant and non-pregnant mares, with a good fit confirmed by the Hosmer-Lemeshow test (χ^2 = 11.449, P= 0.120). It explained 22.8% to 32.0% of the variance (Cox and Snell R^2 and Nagelkerke R^2, respectively) and correctly classified 75.0% of cases.

The predictors included in the model were PG treatment (vs. spontaneous estrus), ovulatory treatment (GnRH, hCG, control), PBIE, rebreeding status, and stallion used for breeding. The detailed logistic regression outcomes for these variables are summarized in Table I. Detailed results are provided below.

Effect of PG treatment for estrus induction on pregnancy outcome

There was no significant effect of estrus induction by prostaglandin treatment on pregnancy outcomes in donkey mares. Twenty-eight donkey mares were treated with PG, and thirty-two mares exhibited estrus after spontaneous luteolysis. The pregnancy rate for PG-treated donkey mares and those exhibiting natural estrus was 57.10% and 78.10%, respectively. The difference was statistically non-significant (*P*= 0.307).

Table I. Effect of estrus induction, ovulatory treatment, reproductive status, and stallion used on pregnancy outcome in donkey mares.

Param-eter	n	Pregnancy outcome positive (%)	*P* value	Odds ratio (OR)	95% C.I. for EXP (B)	
					Lower	Upper
PG treatment						
Yes	28	16 (57.10%)		Reference		
No	32	25 (78.10%)	0.307	1.930	0.547	6.805
Ovulation treatment						
GnRH	20	15 (75%)	0.100	Reference		
hCG	20	16 (80%)	0.075	0.220	0.042	1.162
Control	20	10 (50%)	**0.048**	0.185	0.035	0.987
PBIE status						
Yes	8	6 (75%)		Reference		
No	52	35 (67.30%)	0.486	0.463	0.24	18.897
Yes	14	8 (57.10%)		Reference		
No	46	33 (80.50%)	0.307	2.557	0.422	15.472
Stallion used						
Stallion-1	23	14 (60.90%)	0.067	Reference		
Stallion-2	19	11 (57.90%	**0.034**	9.225	1.185	71.834
Stallion-3	18	16 (88.90%	**0.024**	13.130	1.396	123.47

Values in bold indicate statistical significance at $P < 0.05$. Model fit: –2 Log likelihood, 59.387; $\chi^2(7)$, 15.532; P, 0.030 (Omnibus test). Hosmer-Lemeshow χ^2, 11.449; df, 7; P, 0.120. PG, Prostaglandin; PBIE, Post-breeding induced endometritis; OR, Odds ratio; CI, Confidence interval. GnRH, gonadotropin-releasing hormone; hCG, human chorionic gonadotropin.

Effect of ovulation induction treatment on pregnancy outcome

There was a significant impact of ovulation induction by hormonal treatment on pregnancy outcome as compared to the control group. When compared with the GnRH and hCG groups, the control group showed a lower likelihood of successful pregnancy outcomes (OR= 0.185, 95% CI [0.035, 0.987], *P* = 0.048). However, no significant association was detected between the hCG group and the GnRH group (OR= 0.220, 95% CI [0.042, 1.162], *P*= 0.075).

Effect of PBIE on pregnancy outcome

There were only eight mares that exhibited PBIE. The PBIE group was treated with oxytocin just after ovulation, and no harmful effect was observed on pregnancy outcome when compared with non-PBIE mares (n= 52). The difference was statistically non-significant (*P*= 0.486).

Effect of rebreeding on pregnancy outcome

There was no statistically significant effect (*P* = 0.307) of rebreeding on pregnancy outcomes in donkey mares. The pregnancy rate in the rebreeding and single-breeding groups was 57.10% and 80.50%, respectively.

Effect of the stallion used for breeding on pregnancy outcome

The analysis also highlighted the role of stallion type as a significant predictor. Stallion-2 (OR = 9.225, 95% CI [1.185, 71.834], *P* = 0.034) and Stallion-3 (OR = 13.130, 95% CI [1.396, 123.470], *P* = 0.024) were associated with a higher likelihood of successful pregnancy outcomes compared to Stallion-1. Overall, the pregnancy rates for Stallion-1, Stallion-2, and Stallion-3 were 60.90%, 57.90%, and 88.90%, respectively.

Effect of different hormonal treatments on ovulation

Ovulation rates differed across treatment groups, with 100% of mares ovulating in both the GnRH and hCG groups, compared to 85% in the control group. This association was statistically significant ($\chi^2(2, N= 60) = 6.316$, $P = 0.043$), indicating that hormonal treatment was positively associated with ovulation success.

A separate chi-square test assessed the relationship between ovulation and PG treatment. Ovulation occurred in 89.3% of PG-treated mares and 100% of mares with spontaneous estrus (Table II). Although this difference approached significance ($\chi^2(1, N = 60) = 3.609$, $P = 0.057$), Fisher's Exact Test yielded a P-value of 0.096, suggesting the result should be interpreted with caution.

Table II. Association between ovulation and hormonal treatment groups in donkey mares.

Variables	Ovulation		Chi-square *P*-Value
	Yes count (Row N %)	No count (Row N %)	
Group			
GnRH	20 (100%)	0	**0.043**
hCG	20 (100%)	0	
Control	17 (85%)	3(15%)	
PG			
Yes	25 (89.30%)	3 (10.70%)	0.057
No	32 (100%)	0	

Note: Values in bold indicate statistical significance at α = 0.05. Fisher's Exact Test for PG treatment yielded *P* = 0.096. For other abbreviations, see Table I.

Effect of different hormonal treatments on the size of follicle

A one-way ANOVA was conducted to compare the effect of different treatments (GnRH, hCG, Control) on the size of ovulatory follicles of the donkey mares (Table III). The statistical analysis revealed that there were no significant differences in the size of the ovulatory follicle across the three groups ($P > 0.05$).

Table III. Comparison of size of ovulatory follicle across treatment types (GnRH, hCG, and control).

Treat-ment type	N	Mean	SD	SEM	95% CI for mean		ANOVA
					Lower bound	Upper bound	
GnRH	20	42.5	2.26	0.51	41.45	43.56	F(2, 57) = 2.318, *P*=0.108
hCG	20	41.4	1.73	0.39	40.59	42.21	
Control	20	41.3	1.84	0.42	40.44	42.17	

No statistically significant difference was observed among treatment groups (one-way ANOVA: $F(2, 57) = 2.318$, $P = 0.108$). SD, standard deviation; SEM, standard error of the mean; CI, confidence interval. For other abbreviations, see Table I.

DISCUSSION

The present study investigated the effect of different hormonal treatments, reproductive conditions, and stallion types on ovulatory responses and pregnancy outcomes in donkey mares. While ovulation rates were significantly influenced by hormonal treatments, pregnancy outcomes were most strongly associated with both ovulation induction and stallion identity. Other factors, such as follicle size, rebreeding, and post-breeding endometritis showed no significant effect. The findings provide valuable insights into the reproductive management of donkey mares, particularly in subtropical climates such as Pakistan.

The overall pregnancy rate observed in this study was 68.33%, with GnRH and hCG treatments significantly enhancing pregnancy rates compared to the control group. Specifically, the pregnancy rates for GnRH (75.00%) and hCG (80.00%) treatments were notably higher than the control group's rate of 50.00% ($P = 0.048$). These results align with previous studies that demonstrate the efficacy of both GnRH and hCG in improving ovulation rates and subsequent pregnancy outcomes (McCue *et al.*, 2007; Segabinazzi *et al.*, 2021). A recent study from Pakistan has reported no difference in the ovulation rate of mares treated with GnRH or hCG (Mahmood *et al.*, 2024). Furthermore, the findings of our study reflect a growing body of evidence suggesting that these hormonal treatments can effectively synchronize ovulation with Breeding, especially in species where reproductive seasonality plays a lesser role, such as donkeys. This finding is in alignment with current studies from Pakistan, which suggest that seasonality does not cause very strict breeding patterns in thoroughbred and Arab mares under subtropical conditions of Pakistan (Mahmood *et al.*, 2024).

The lack of significant differences between the GnRH and hCG groups ($P = 0.075$) in our study suggests that both treatments are similarly effective for inducing ovulation in donkey mares, a finding consistent with studies on equine reproductive physiology (Morel, 2020). However, a key point of differentiation is the faster response to hCG treatment, which could offer practical advantages in breeding programs where precise timing is critical (Teixeira *et al.*, 2020).

The analysis of the influence of PG for estrus induction revealed no significant effect on pregnancy outcomes compared to natural estrus ($P = 0.307$). While PG-treated mares had a lower pregnancy rate (57.10%) compared to mares exhibiting natural estrus (78.10%), the difference was not statistically significant. This suggests that PG, while effective in inducing estrus, may not always lead to improved pregnancy outcomes, especially when compared to natural estrus cycles. These results underscore the importance of optimizing the timing and selection of mares for hormonal treatments to maximize breeding success. However, PG treatment was effective in inducing estrus induction and attaining a reasonable pregnancy rate. These results were in alignment with previous studies which suggest the use of PG in CL having mares (Piotrowska-Tomala *et al.*, 2022; Segabinazzi *et al.*, 2021; Urge *et al.*, 2022).

Additionally, PBIE was observed in 13.3% of the mares, but treatment with oxytocin did not significantly affect pregnancy outcomes ($P = 0.486$). This finding aligns with existing literature that suggests that while PBIE is a common reproductive challenge, timely intervention with oxytocin can mitigate its impact on pregnancy outcomes (Ortega-Ferrusola *et al.*, 2022; Woodward and Troedsson, 2013). The non-significant difference between single-breeding (80.50%) and rebreeding groups (57.10%) ($P = 0.307$) suggests that rebreeding does not substantially enhance pregnancy rates, highlighting the importance of successful initial breeding attempts for improved reproductive efficiency. This suggests that timed breeding of donkey mares after hormonal treatment for ovulation induction may reduce breeding stress without compromising the pregnancy rate. This finding is in alignment with previous studies that highlight the importance of timed breeding in donkey mares (Fanelli *et al.*, 2019; Yang *et al.*, 2021).

The choice of stallion played a significant role in determining pregnancy outcomes, with Stallion-2 and Stallion-3 showing significantly higher pregnancy rates (P = 0.034 and P = 0.024, respectively) than Stallion-1. These results highlight the critical influence of genetic and physiological factors on breeding success, reinforcing the need for careful stallion selection in breeding programs. This finding is in accordance with previous studies that highlight the importance of the breeding soundness and fertility of stallions in equine breeding programs (Aissanou *et al*., 2022; Johannisson *et al*., 2023). Future research could explore the underlying genetic or physiological characteristics that make certain stallions more successful, providing deeper insights into breeding management.

Regarding follicular size, there was no significant difference between treatment groups (P=0.108), with mean follicle sizes ranging from 41.30 mm to 42.50 mm. This finding, along with the high ovulation rates in the GnRH and hCG groups (100%), suggests that while follicle size can be a useful indicator of reproductive status, it may not be the sole determinant of pregnancy success. Other factors, such as the timing of ovulation and the quality of the oocyte, likely play more pivotal roles. However, the size of the ovulatory follicle described in our study may be used as an important indicator for the timed breeding of American mammoth donkey mares under subtropical conditions. A similar study from Pakistan has reported that each breed of mare has unique size of ovulatory follicle under subtropical conditions of Pakistan (Mahmood *et al*., 2024b).

Finally, the lack of significant seasonal or monthly variations in breeding and foaling rates (P>0.05) indicates that donkey mares exhibit consistent reproductive performance throughout the year, even under subtropical conditions. This consistency contrasts with species that exhibit more pronounced seasonal breeding patterns (Vilhanová *et al*., 2021); however, it aligns with another study about the follicular dynamics of Jennies over an entire year (Li *et al*., 2021) and suggests that donkey breeding programs in Pakistan could potentially operate year-round with optimized hormonal treatments.

CONCLUSION

This study demonstrates that American Mammoth Jackstock donkey mares exhibit consistent reproductive activity throughout the year under subtropical conditions in Pakistan. Hormonal treatments with GnRH and hCG significantly enhanced both ovulation and pregnancy rates, confirming their effectiveness in improving reproductive efficiency. While stallion selection appeared to influence fertility outcomes, no significant effects were observed from estrus induction with prostaglandin, PBIE, or rebreeding practices. These findings offer practical guidance for optimizing breeding strategies in donkeys under subtropical management systems.

DECLARATIONS

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IRB approval

This study was approved by the Board of Advanced Studies and Research (BASR) of Allama Iqbal Open University (AIOU) under notification No. F.6-9/2022-basr/6903.

Ethical statement

All the procedures were performed in accordance with the ARRIVE guidelines (Percie du Sert *et al*., 2020), and ethical approval was obtained from the University's Animal Welfare and Ethics Committee.

Availability of data

Upon a reasonable request, the datasets of this study can be available from the first and corresponding author.

Statement of conflict of interest

The authors declare no conflict of interest that might affect the impartiality of this article.

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