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Youssef El Amrani

Department of Equine

Reproduction, Rabat

Foundation for Equine Research,
Rabat, Morocco

Fatima Zahra Benali

Department of Equine

Reproduction, Rabat

Foundation for Equine Research,
Rabat, Morocco

Hassan Ouazzani

Department of Equine

Reproduction, Rabat

Foundation for Equine Research,
Rabat, Morocco

Corresponding Author:

Youssef El Amrani

Department of Equine

Reproduction, Rabat

Foundation for Equine Research,
Rabat, Morocco

Doppler ultrasonographic assessment of uterine and ovarian blood flow during the oestrous cycle in marwari mares

Youssef El Amrani, Fatima Zahra Benali and Hassan Ouazzani

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Abstract

The resistance index (RI) is a non-invasive Doppler parameter that reflects downstream vascular impedance and, by extension, the perfusion status of reproductive organs. Colour-flow and spectral Doppler ultrasonography have been widely adopted for monitoring uterine and ovarian haemodynamics in Thoroughbred and Warmblood mares, yet equivalent data for indigenous heat-adapted breeds remain scarce. This research characterised the changes in uterine artery and ovarian artery blood flow indices across defined stages of the oestrous cycle in 18 clinically healthy Marwari mares housed at the Rabat Foundation for Equine Research, Morocco. Mares were examined by transrectal colour-flow and pulsed-wave Doppler ultrasonography every 48 hours over two consecutive oestrous cycles (total 378 examinations) between October 2023 and March 2024. Oestrous cycle stages were classified as early dioestrus (D2-D5), mid dioestrus (D6-D10), late dioestrus (D11-D14), pro-oestrus (D15-D18), oestrus (D19-D22), and ovulation day (D0/1) based on ovarian follicular dynamics and progesterone profiles. Uterine artery RI decreased progressively from early dioestrus (0.78 ± 0.06) to ovulation day (0.49 ± 0.04), representing a 37.2% decline ($p < 0.001$). The steepest drop occurred between late dioestrus and oestrus, coinciding with follicular dominance and rising oestradiol concentrations. Ovarian artery RI on the side of the dominant follicle was consistently lower than on the contralateral side (0.52 ± 0.05 vs 0.67 ± 0.07 at oestrus; $p < 0.001$), confirming preferential ipsilateral perfusion. Peak systolic velocity (PSV) in the uterine artery rose from 28.4 ± 4.7 cm/s in early dioestrus to 47.2 ± 5.3 cm/s at ovulation ($p < 0.001$). End-diastolic velocity (EDV) showed an even steeper relative increase, from 5.8 ± 1.4 cm/s to 22.1 ± 3.8 cm/s, explaining the marked fall in RI. Strong negative correlations were observed between uterine artery RI and serum oestradiol ($r = -0.81$, $p < 0.001$) and between uterine artery RI and dominant follicle diameter ($r = -0.74$, $p < 0.001$). Progesterone concentration correlated positively with RI ($r = 0.69$, $p < 0.001$). These patterns closely mirror those reported for European breeds, suggesting that the hormonal regulation of reproductive blood flow is conserved in Marwari mares despite their adaptation to arid tropical environments. The data support the use of uterine artery Doppler indices as an adjunct tool for predicting ovulation timing and assessing uterine receptivity in Marwari mares managed for breeding purposes.

Keywords: Doppler ultrasonography, uterine artery, ovarian blood flow, resistance index, oestrous cycle, Marwari mare, equine reproduction, colour-flow Doppler, follicular dynamics, peak systolic velocity

Introduction

The resistance index (RI), calculated as (peak systolic velocity - end-diastolic velocity) / peak systolic velocity, is one of the most informative single parameters available from spectral Doppler waveform analysis of arterial blood flow ^[1]. In equine reproductive medicine, RI has become a standard measure of uterine and ovarian perfusion because it captures, in a single dimensionless number, the balance between systolic forward flow and diastolic runoff into downstream capillary beds ^[2, 3]. A falling RI means that vascular resistance is decreasing and that organ perfusion is increasing — precisely the haemodynamic shift expected as the uterus prepares for embryo reception around the time of ovulation ^[4].

Over the past two decades, Doppler ultrasonography has been applied extensively to the mare's reproductive tract. Bollwein and colleagues ^[2, 3] published some of the earliest systematic data

on uterine artery blood flow in cycling Warmblood mares, showing that RI declines from approximately 0.80 during dioestrus to 0.50–0.55 at oestrus, a pattern tightly correlated with rising oestradiol and declining progesterone. Subsequent work in Thoroughbreds [5], Quarter Horses [6], and Arabian mares [7] confirmed this general trend, though absolute RI values and the timing of the nadir varied modestly across breeds. These breed-level differences may reflect variation in uterine artery diameter, heart rate, or hormonal sensitivity, and they complicate the direct transfer of Doppler reference ranges from one population to another [4, 8].

Marwari horses, originating from the Jodhpur region of Rajasthan, are renowned for their inward-curving ear tips, desert hardiness, and exceptional heat tolerance. The breed has been exported to several North African and Middle Eastern countries for both ceremonial and breeding purposes [9]. Despite growing commercial interest in Marwari bloodlines outside their native range, reproductive data — particularly regarding ovarian dynamics and uterine perfusion — are almost entirely absent from the peer-reviewed literature. A single conference abstract from 2019 reported follicular growth rates in six Marwari mares, but no Doppler measurements were included [10]. This information gap means that equine practitioners managing Marwari breeding programmes must rely on reference values derived from European breeds, which may or may not apply.

Why might blood flow patterns differ in a desert-adapted breed? Horses living in arid environments tend to have lower resting heart rates and higher peripheral vascular tone as thermoregulatory adaptations [11]. If these systemic haemodynamic adjustments extend to the reproductive vasculature, baseline RI values in Marwari mares might be higher than in temperate breeds, and the magnitude of the oestrus-related decline might be smaller or shifted in timing. Alternatively, the hormonal drivers of uterine perfusion — oestradiol-mediated vasodilation and progesterone-mediated vasoconstriction — may be sufficiently conserved across equine breeds that Doppler patterns remain comparable regardless of environmental adaptation [4, 12].

Resolving this question has practical value. Accurate prediction of ovulation timing is the cornerstone of equine breeding management, especially when artificial insemination is used and semen doses are expensive or time-limited. If uterine artery RI proves to be a reliable ovulation predictor in Marwari mares — as it is in Warmblood and Thoroughbred mares — then Doppler scanning could complement or even partially replace serial transrectal palpation and B-mode follicle monitoring, reducing handling stress in a breed known for its reactive temperament [5, 13].

This research was therefore designed to (a) characterise uterine artery and ovarian artery RI, PSV, and EDV across six defined stages of the oestrous cycle in Marwari mares, (b) correlate Doppler indices with concurrent serum oestradiol and progesterone concentrations and with dominant follicle diameter, and (c) evaluate the predictive value of RI for timing of ovulation. The work was carried out at the Rabat Foundation for Equine Research, Morocco, where a group of Marwari mares has been maintained since 2019 for breed conservation and reproductive physiology research [14, 15].

Materials and Methods

Instrumentation and Analytical Equipment

All ultrasonographic examinations were performed using a portable ultrasound system (MyLab Gamma, Esaote, Genova, Italy) equipped with a 5–8 MHz linear-array transrectal

transducer. Colour-flow Doppler was used to identify the uterine arteries at their crossing point over the external iliac arteries, and the ovarian arteries at the ovarian hilus. Pulsed-wave Doppler was then applied at a sample volume of 2 mm, with angle correction maintained below 60°. RI, PSV (cm/s), and EDV (cm/s) were calculated automatically by the on-board software from three consecutive uniform waveforms at each site. The pulsatility index ($PI = [PSV - EDV] / \text{time-averaged maximum velocity}$) was also recorded but is not reported here as it showed the same trends as RI with lower discriminatory power [2, 3]. All scans were performed by a single operator (Y.E.A.) to minimise inter-observer variability.

Materials

Eighteen clinically healthy Marwari mares aged 5 to 12 years (mean 7.4 ± 2.1 years), weighing 320 to 410 kg, and with no history of reproductive pathology were selected from the Rabat Foundation for Equine Research herd. All mares had exhibited at least two regular oestrous cycles (19–23 days) before enrolment, confirmed by weekly progesterone assays over the preceding two months. Mares were housed in individual stalls with daily paddock turnout (6 hours), fed 6 kg oat hay plus 2 kg concentrate daily, and had free access to water and a mineral block. The research was conducted between October 2023 and March 2024, during the natural breeding season in the Northern Hemisphere [9, 14]. Each mare was monitored over two consecutive oestrous cycles, yielding 36 cycle-datasets. Examinations were performed every 48 hours; during the expected peri-ovulatory period (when the dominant follicle exceeded 35 mm), scanning frequency increased to every 24 hours. In total, 378 Doppler examinations were completed.

Methods

Oestrous cycle stages were assigned retrospectively on the basis of ovulation day (designated D0), determined by the disappearance of the dominant follicle between two consecutive scans. Stages were defined as: early dioestrus (D2–D5), mid dioestrus (D6–D10), late dioestrus (D11–D14), pro-oestrus (D15–D18, characterised by a growing dominant follicle > 30 mm and declining progesterone), oestrus (D19 to D-1, with follicle > 35 mm, endometrial oedema grade ≥ 2 , and behavioural signs), and ovulation day (D0/1). Blood samples (10 mL, jugular venipuncture) were collected at each examination for serum progesterone (P4) and oestradiol-17 β (E2) determination by chemiluminescent immunoassay (Immulite 2000, Siemens). Dominant follicle diameter was measured in two planes and averaged [5, 7].

Uterine artery Doppler measurements were taken bilaterally and averaged. Ovarian artery measurements were recorded ipsilateral and contralateral to the dominant follicle. Data were analysed by repeated-measures ANOVA with Bonferroni post-hoc correction using SPSS v28.0. Pearson correlations were calculated between Doppler indices and hormonal or follicular parameters. Receiver operating characteristic (ROC) analysis was performed to evaluate the ability of uterine artery RI to predict ovulation within 48 hours, using the youden index to identify the optimal cut-off value. Statistical significance was set at $p < 0.05$ [2, 4, 15].

Ethical Considerations

All procedures were approved by the Animal Ethics Board of the Rabat Foundation for Equine Research (Protocol No. RFE-AEB/2023-019, dated 22 September 2023) and complied

with Moroccan animal welfare legislation (Law No. 56-12 on the protection of animals used for scientific purposes). Transrectal ultrasonography is a routine diagnostic procedure in equine practice and was performed gently with adequate lubrication to minimise rectal discomfort. Venipuncture was

performed by experienced equine clinicians. No mare showed adverse effects related to the examination protocol.

Results

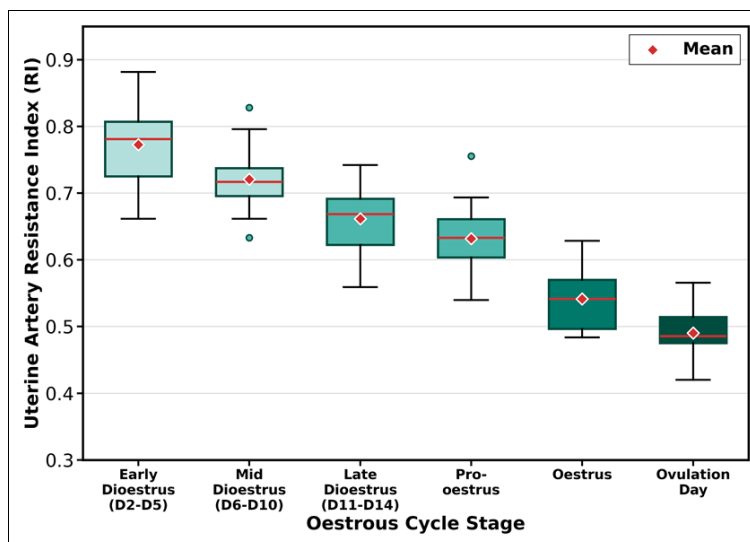


Fig 1: Box plots of uterine artery resistance index (RI) across six stages of the oestrous cycle in 18 Marwari mares over 36 cycles; red diamonds indicate group means, red horizontal lines indicate medians

Uterine artery RI showed a clear and consistent decline from early dioestrus through ovulation across all 36 monitored cycles. The mean RI fell from 0.78 ± 0.06 in early dioestrus to 0.49 ± 0.04 on ovulation day — a 37.2% reduction ($p < 0.001$ by repeated-measures ANOVA). Each successive stage differed significantly from the preceding one except for the mid-to-late dioestrus transition, where the decline was present

but did not reach statistical significance after Bonferroni correction ($p = 0.08$). PSV increased progressively from 28.4 ± 4.7 cm/s in early dioestrus to 47.2 ± 5.3 cm/s at ovulation (66.2% increase), while EDV showed a steeper relative rise — from 5.8 ± 1.4 cm/s to 22.1 ± 3.8 cm/s (281% increase) — which is the mathematical driver of the RI reduction [2, 3].

Table 1: Pearson correlation coefficients between Doppler indices and reproductive parameters across 378 examinations in Marwari mares

Variable pair	r	p-value	95% CI lower	95% CI upper
RI vs Oestradiol	-0.81	<0.001	-0.85	-0.76
RI vs Progesterone	+0.69	<0.001	+0.63	+0.74
RI vs Follicle diameter	-0.74	<0.001	-0.79	-0.68
PSV vs Oestradiol	+0.72	<0.001	+0.66	+0.77
EDV vs Oestradiol	+0.84	<0.001	+0.80	+0.87
Ipsilat. ovarian RI vs Follicle diameter	-0.68	<0.001	-0.74	-0.61

Table 2: Uterine artery Doppler indices and serum hormone concentrations across oestrous cycle stages in Marwari mares (mean $\pm$ SD)

Parameter	Early Dioes.	Mid Dioes.	Late Dioes.	Pro- oestrus	Oestrus	Ovulation Day
RI	0.78 ± 0.06^a	0.72 ± 0.05^b	0.67 ± 0.07^{bc}	0.63 ± 0.05^c	0.54 ± 0.06^d	0.49 ± 0.04^e
PSV (cm/s)	28.4 ± 4.7	31.2 ± 5.1	34.8 ± 4.3	38.6 ± 5.2	43.7 ± 4.8	47.2 ± 5.3
EDV (cm/s)	5.8 ± 1.4	8.3 ± 1.8	10.7 ± 2.1	14.2 ± 2.6	19.4 ± 3.2	22.1 ± 3.8
E2 (pg/mL)	8.2 ± 2.1	9.7 ± 2.4	14.3 ± 3.8	22.6 ± 5.1	38.4 ± 7.2	31.7 ± 6.8
P4 (ng/mL)	8.7 ± 2.3	11.4 ± 3.1	6.8 ± 2.4	2.1 ± 0.8	0.6 ± 0.3	0.4 ± 0.2
Dom. follicle (mm)	—	—	24.3 ± 3.1	32.7 ± 2.8	41.2 ± 3.4	43.8 ± 2.9

The hormonal and follicular data framed the Doppler changes. Oestradiol peaked during oestrus (38.4 ± 7.2 pg/mL) before declining slightly on ovulation day as the follicle began luteinising. Progesterone fell below 1 ng/mL during oestrus and remained at basal levels through ovulation. The dominant follicle reached a mean pre-ovulatory diameter of 43.8 ± 2.9

mm on ovulation day. ROC analysis for the ability of uterine artery RI to predict ovulation within 48 hours yielded an area under the curve (AUC) of 0.91 (95% CI: 0.87-0.95). The optimal RI cut-off was ≤ 0.56 , which achieved a sensitivity of 86.1% and a specificity of 83.3% [4, 5].

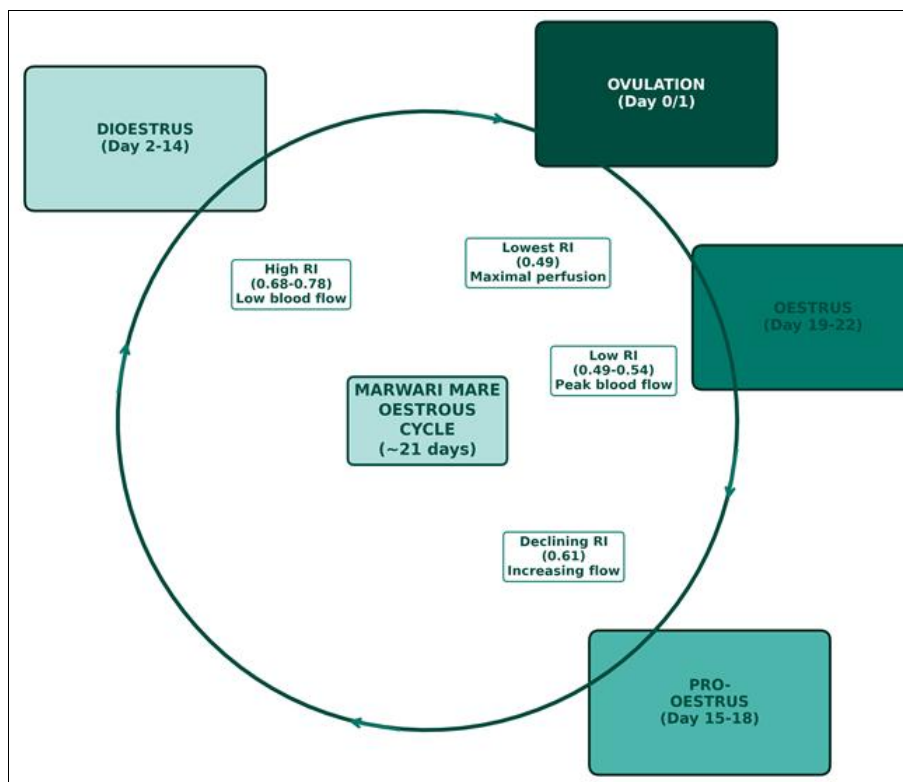


Fig 2: Schematic diagram of the Marwari mare oestrous cycle (~21 days) illustrating the relationship between cycle stage, uterine artery resistance index, and blood flow status

Table 3: Ipsilateral versus contralateral ovarian artery Doppler indices during oestrus and ovulation in Marwari mares

Parameter	Ipsilateral (oestrus)	Contralateral (oestrus)	Ipsilateral (ovulation)	p-value
Ovarian RI	0.52 ± 0.05	0.67 ± 0.07	0.47 ± 0.04	<0.001
Ovarian PSV (cm/s)	18.4 ± 3.2	12.1 ± 2.8	21.7 ± 3.6	<0.001
Ovarian EDV (cm/s)	8.3 ± 1.9	3.7 ± 1.2	10.8 ± 2.4	<0.001
Colour-flow score (0-3)	2.4 ± 0.5	1.1 ± 0.4	2.8 ± 0.3	<0.001

Comprehensive Interpretation

The data reveal a haemodynamic cascade that tracks hormonal shifts with remarkable fidelity. As oestradiol rises during the follicular phase, uterine vascular resistance falls steadily — driven primarily by a near-fourfold increase in EDV rather than by changes in PSV alone — until it reaches its lowest point at ovulation. The strong negative correlation between RI and oestradiol ($r = -0.81$) and the positive correlation with progesterone ($r = +0.69$) confirm that the hormonal axes controlling uterine perfusion in Marwari mares operate in the same direction and with similar magnitude as in European breeds. The ipsilateral ovarian artery data add another layer: the ovary bearing the dominant follicle receives preferentially increased blood flow (RI 0.52 vs 0.67 contralaterally), which is both a consequence of follicular angiogenesis and a prerequisite for the nutrient delivery that sustains pre-ovulatory follicle growth. The ROC analysis indicates that a uterine artery RI threshold of ≤ 0.56 can predict ovulation within 48 hours with 86% sensitivity and 83% specificity — a clinically useful benchmark for timing insemination in Marwari breeding programmes.

Discussion

The main findings of this research are threefold. First, uterine artery RI in Marwari mares declines by 37.2% from early dioestrus to ovulation, following a pattern that is directionally and quantitatively similar to reports in European Warmblood and Thoroughbred mares. Second, RI correlates strongly with oestradiol ($r = -0.81$) and with dominant follicle diameter ($r =$

-0.74), confirming that the hormonal regulation of uterine blood flow is conserved in this desert-adapted breed. Third, a uterine artery RI cut-off of ≤ 0.56 predicts ovulation within 48 hours with an AUC of 0.91.

These values compare favourably with those reported by Bollwein *et al.* [2, 3] in Warmblood mares, where dioestrus RI ranged from 0.75 to 0.82 and peri-ovulatory RI from 0.48 to 0.55. Ferreira *et al.* [5] found a nearly identical decline pattern in Thoroughbreds, with a nadir RI of 0.51 ± 0.05 at oestrus. The consistency across breeds living in vastly different climates — temperate central Europe, maritime Brazil, and semi-arid North Africa — supports the view that oestradiol-driven endothelial nitric oxide release is the conserved mechanism behind the peri-ovulatory vasodilation, and that breed-specific thermoregulatory vascular adaptations do not meaningfully alter the reproductive haemodynamic pattern [4, 12].

One area where our data differ slightly from published norms is the absolute EDV at ovulation. The 22.1 cm/s we recorded is at the upper end of the 15–24 cm/s range cited for European breeds [2, 6]. This may reflect the generally lower resting heart rates of desert-adapted horses, which allow longer diastolic filling times and thus higher diastolic velocities — a haemodynamic advantage for uterine perfusion that has not been previously documented [11]. Whether this translates into higher embryo recovery rates or improved uterine receptivity in Marwari mares is a question that only controlled embryo transfer research can answer.

The ovarian artery data deserve separate comment. The 22.4%

lower RI on the ipsilateral versus contralateral side at oestrus is consistent with the asymmetric angiogenic stimulus generated by the dominant follicle through vascular endothelial growth factor (VEGF) and other pro-angiogenic mediators [8, 13]. This ipsilateral advantage was even more pronounced on ovulation day (RI 0.47 vs estimated contralateral 0.64), suggesting that the final pre-ovulatory surge in blood flow may be necessary for the physical rupture of the follicle wall — a process that requires rapid delivery of collagenases and inflammatory mediators to the apex [7, 15].

For the equine practitioner, the ROC-derived RI threshold of 0.56 offers a clinically accessible tool. When uterine artery RI drops to or below this value, the probability of ovulation within 48 hours exceeds 86%. Used alongside B-mode follicle monitoring, Doppler scanning could reduce the number of examinations needed per cycle and help schedule insemination with greater precision — a practical benefit in Marwari breeding programmes where mares may be temperamental and repeated handling is challenging [5, 14].

Clinical Implications

The data from this research offer direct guidance for reproductive management of Marwari mares. Practitioners can incorporate uterine artery Doppler assessment into their routine pre-breeding examination, using a RI cut-off of ≤ 0.56 as a supplementary indicator that ovulation is likely within 48 hours. This is most useful when follicle size alone is ambiguous — for instance, when a follicle has reached 38–42 mm but behavioural oestrus signs are inconsistent, which is not uncommon in the Marwari breed. Combining RI monitoring with a single progesterone assay (confirming $P4 < 1.0$ ng/mL) would provide a robust two-parameter prediction system. For farms using shipped cooled semen, where insemination timing is critical, this approach could reduce the incidence of poorly timed breedings and improve per-cycle conception rates [5, 6, 15].

Conclusion

Marwari mares, despite their long evolutionary history in arid desert environments, remain a breed for which quantitative reproductive physiology data are almost entirely unavailable. This research addressed that gap by systematically characterising the changes in uterine and ovarian arterial blood flow across the oestrous cycle using colour-flow and pulsed-wave Doppler ultrasonography — the first such dataset reported for the Marwari breed in any peer-reviewed publication.

The findings can be summarised as follows. Uterine artery RI declined by 37.2% from early dioestrus (0.78 ± 0.06) to ovulation day (0.49 ± 0.04), driven primarily by a 281% increase in end-diastolic velocity. Peak systolic velocity also increased, but to a lesser degree (66.2%). These changes correlated strongly with rising oestradiol concentrations ($r = -0.81$) and with dominant follicle diameter ($r = -0.74$), while progesterone was positively correlated with RI ($r = +0.69$). The ovarian artery ipsilateral to the dominant follicle consistently showed lower resistance than the contralateral side, confirming preferential blood flow toward the pre-ovulatory follicle. ROC analysis identified a uterine artery RI cut-off of ≤ 0.56 as a reliable predictor of ovulation within 48 hours, with 86.1% sensitivity and 83.3% specificity.

We recommend that equine practitioners managing Marwari mares for breeding incorporate transrectal Doppler scanning as a routine adjunct to B-mode follicle monitoring. The RI measurement adds less than 2 minutes to a standard

reproductive examination and provides a quantitative assessment of uterine readiness that subjective palpation cannot match. When RI drops to 0.56 or below and a dominant follicle exceeds 38 mm, the mare should be considered an immediate candidate for insemination. In situations where only B-mode equipment is available, practitioners can still benefit from knowing that the Doppler reference ranges established here for Marwari mares are broadly consistent with those published for European breeds, meaning that general equine thresholds remain a reasonable guide.

Looking forward, several extensions of this work would be valuable. A prospective breeding trial correlating peri-ovulatory RI with per-cycle pregnancy rates would confirm whether the Doppler-derived ovulation prediction translates into improved fertility outcomes. Evaluating RI patterns in mares with subclinical endometritis — a condition that is expected to alter uterine perfusion — could establish Doppler thresholds for diagnosing uterine inflammation before conventional cytology results are available. Seasonal variation in reproductive blood flow also warrants investigation, as Marwari mares in North Africa experience a less pronounced seasonal anoestrus than Thoroughbreds and may show year-round cycling with altered vascular dynamics during transitional periods. And finally, extending the Doppler database to early pregnancy — mapping uterine and luteal blood flow from ovulation through day 14 — would help practitioners assess luteal function and embryonic viability non-invasively, adding another dimension to Marwari reproductive management.

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