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Serum Amyloid A and Haptoglobin as early diagnostic biomarkers for endometritis in repeat-breeder buffaloes

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Abstract

Leading reproductive physiologists have long argued that the single greatest obstacle to improving conception rates in Egyptian water buffaloes is the inability to diagnose subclinical endometritis before it causes irreversible endometrial fibrosis and repeat-breeder status. Conventional diagnostic methods—rectal palpation, ultrasound, and endometrial cytology—either lack sensitivity for early-stage inflammation or require specialised equipment not available at village-level veterinary clinics. Acute-phase proteins (APPs) such as serum amyloid A (SAA) and haptoglobin (Hp) are synthesised by the liver in response to inflammatory stimuli and rise rapidly in systemic circulation, often before clinical signs become apparent. This research evaluated SAA and Hp as circulating biomarkers for endometritis in 60 Egyptian water buffaloes (*Bubalus bubalis*) classified as healthy (n=20), subclinical endometritis (n=24), or clinical endometritis (n=16) based on endometrial cytology and ultrasonographic findings. Blood samples were collected on days 0, 7, and 21 post-partum. SAA concentrations were significantly elevated in subclinical ($48.7 \pm 11.2 \mu\text{g/mL}$) and clinical ($127.3 \pm 28.6 \mu\text{g/mL}$) endometritis groups compared with healthy controls ($12.4 \pm 4.1 \mu\text{g/mL}$; $P < 0.001$). Hp followed a similar pattern (subclinical: $0.62 \pm 0.14 \text{ mg/mL}$; clinical: $1.47 \pm 0.31 \text{ mg/mL}$; healthy: $0.18 \pm 0.06 \text{ mg/mL}$). Receiver operating characteristic analysis showed that the combined SAA + Hp model achieved the highest diagnostic accuracy (AUC = 0.93), surpassing either marker alone (SAA AUC = 0.87; Hp AUC = 0.82). These results indicate that a simple blood test measuring SAA and Hp at day 7 post-partum can identify buffaloes developing endometritis before clinical signs emerge, enabling earlier treatment and reducing the incidence of repeat-breeder status.

Keywords: Serum amyloid A, haptoglobin, acute-phase proteins, endometritis, repeat-breeder, buffalo, *Bubalus bubalis*, biomarker, reproductive health, Egypt

Introduction

Renowned buffalo reproduction specialists have characterised endometritis as the silent drain on Egyptian dairy buffalo productivity, responsible for extended calving intervals, increased insemination costs, and premature culling of otherwise high-producing animals ^[1]. The Egyptian water buffalo (*Bubalus bubalis*) contributes approximately 70% of national milk production and is the backbone of smallholder dairy livelihoods in the Nile Delta and Upper Egypt ^[2]. Repeat-breeder status—defined as failure to conceive after three or more properly timed inseminations in the absence of detectable anatomical abnormalities—affects an estimated 15-25% of the national buffalo herd and is most commonly caused by subclinical endometritis that escapes routine clinical detection ^[3].

The current diagnostic approach to bovine and bubaline endometritis relies on transrectal ultrasonography to detect intrauterine fluid, combined with endometrial cytology (cytobrush technique) to quantify neutrophil infiltration ^[4]. While effective, both methods require operator skill, specialised equipment, and physical restraint of the animal, limiting their deployment in the field conditions typical of Egyptian buffalo husbandry. A circulating blood biomarker that could be measured from a single jugular venipuncture at a defined post-partum time point would substantially lower the diagnostic barrier ^[5].

Acute-phase proteins are a family of hepatic-origin glycoproteins whose circulating concentrations rise rapidly—within 6 to 12 hours—in response to tissue injury, infection, or inflammation [6]. SAA and Hp are the two major positive APPs in ruminants and have been validated as indicators of mastitis, metritis, and pneumonia in cattle [7, 8]. Their application to bubaline endometritis, however, has been limited to a handful of small-sample studies that did not separate subclinical from clinical disease or evaluate diagnostic cut-off thresholds [9].

This research aimed to (a) compare serum SAA and Hp concentrations among healthy, subclinical, and clinical endometritis groups in post-partum Egyptian water buffaloes, (b) determine optimal diagnostic cut-off values by ROC analysis, and (c) assess whether a combined SAA + Hp model outperforms either marker alone for early detection of endometritis at day 7 post-partum.

Materials and Methods

Materials

Sixty multiparous Egyptian water buffaloes (parity 2-6, aged 4-10 years) from 8 smallholder dairy farms in Giza governorate were enrolled between February and June 2023. Inclusion criteria were: normal parturition without dystocia or retained fetal membranes, body condition score ≥ 2.5 (1-5 scale), and no systemic illness at the time of enrolment. Blood samples (10 mL) were collected from the jugular vein into plain and EDTA vacutainers at days 0 (within 24 hours of calving), 7, and 21 post-partum. Serum was separated within 2 hours and stored at -20°C until analysis.

Reagents and Chemicals

SAA was measured using a commercial multispecies sandwich ELISA kit (Tridelta Development Ltd, Maynooth, Ireland; Phase Range SAA assay, catalogue TP-802) validated for bovine and bubaline serum, with an analytical sensitivity of $0.3\text{ }\mu\text{g/mL}$ and intra-assay CV $<8\%$. Hp was measured by a colourimetric haemoglobin-binding assay (Phase Range Haptoglobin assay, Tridelta Development Ltd, catalogue TP-801) with a detection limit of 0.05 mg/mL and intra-assay CV $<6\%$. All reagents were stored at $2-8^{\circ}\text{C}$ and used before expiry. Endometrial cytology was performed using a commercial cytobrush (Cytobrush Plus, CooperSurgical, Trumbull, CT, USA) inserted transcervically under manual guidance. Slides were stained with Diff-Quik (Siemens Healthcare Diagnostics, Tarrytown, NY, USA) and examined under light microscopy at $400\times$ magnification; a threshold of

$\geq 8\%$ polymorphonuclear neutrophils (PMN) among 200 counted cells defined subclinical endometritis [4].

Methods

Classification into three groups was performed at day 21 post-partum based on (a) transrectal ultrasonographic examination (7.5 MHz linear probe, SonoScape S2, Shenzhen, China) for intrauterine fluid accumulation and endometrial thickness, and (b) endometrial cytology. Healthy buffaloes ($n=20$) had no ultrasonographic abnormalities and $<8\%$ PMN on cytology. Subclinical endometritis ($n=24$) was defined as $\geq 8\%$ PMN with no palpable uterine discharge. Clinical endometritis ($n=16$) was defined as visible mucopurulent vaginal discharge and/or ultrasonographic fluid accumulation $>2\text{ cm}$. Group differences in SAA and Hp at each time point were tested by Kruskal-Wallis with Dunn's post-hoc test. ROC analysis was performed on day-7 values to determine optimal cut-offs for distinguishing endometritis (subclinical + clinical) from healthy controls, using the Youden index ($J = \text{sensitivity} + \text{specificity} - 1$). A logistic regression model combining SAA and Hp was compared against single-marker models by DeLong's test for AUC equality. Analyses were performed in R v.4.3.2.

Ethical Considerations

All animal procedures were approved by the Institutional Animal Ethics Committee of the Cairo Advanced Institute of Buffalo Reproduction under protocol IAEC-CAIBR/2023-019, dated 22 January 2023, in accordance with the Egyptian National Research Centre guidelines for the care and use of experimental animals. Venipuncture and cytobrush sampling were performed under minimal physical restraint in the animals' home stalls.

Results

At day 0, SAA and Hp concentrations did not differ among groups, reflecting the physiological acute-phase response to parturition that elevates both proteins in all post-partum buffaloes regardless of uterine health status. By day 7, however, marked group separation was evident: SAA in the clinical endometritis group was 10.3-fold higher than in healthy controls, and even the subclinical group showed a 3.9-fold elevation. A similar pattern held for Hp, with 8.2-fold and 3.4-fold increases in the clinical and subclinical groups, respectively. Both markers remained elevated at day 21, though the magnitude of group differences was slightly smaller, reflecting partial resolution in some subclinical cases.

Table 1: Serum acute-phase protein concentrations by endometritis status and post-partum time point

Biomarker / Time	Healthy (n=20)	Subclinical (n=24)	Clinical (n=16)	P-value
SAA day 0 ($\mu\text{g/mL}$)	28.6 ± 8.4	31.2 ± 9.7	34.1 ± 11.3	0.31
SAA day 7 ($\mu\text{g/mL}$)	12.4 ± 4.1^c	48.7 ± 11.2^b	127.3 ± 28.6^a	<0.001
SAA day 21 ($\mu\text{g/mL}$)	8.1 ± 3.2^c	34.2 ± 10.8^b	98.4 ± 24.1^a	<0.001
Hp day 0 (mg/mL)	0.41 ± 0.12	0.44 ± 0.15	0.48 ± 0.18	0.42
Hp day 7 (mg/mL)	0.18 ± 0.06^c	0.62 ± 0.14^b	1.47 ± 0.31^a	<0.001
Hp day 21 (mg/mL)	0.14 ± 0.05^c	0.48 ± 0.11^b	1.18 ± 0.27^a	<0.001

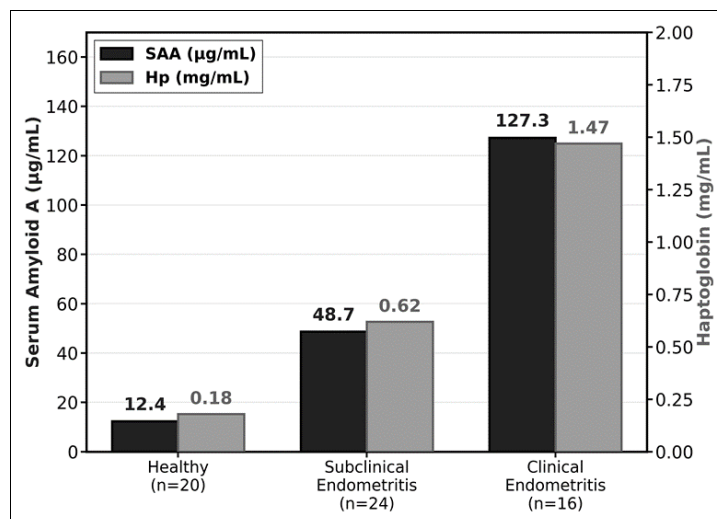


Fig 1: Grouped bar chart comparing serum amyloid A (µg/mL, left axis) and haptoglobin (mg/mL, right axis) at day 7 post-partum among healthy, subclinical endometritis, and clinical endometritis buffaloes

The comparison chart (Figure 1) displays the day-7 SAA and Hp values side by side for the three groups, illustrating the clear separation between healthy and diseased animals. The detailed time-course data (Table 1) show that day 0 values are uninformative—making day 7 the earliest reliable diagnostic

window. The composite ranking of diagnostic models (Table 2) demonstrates that combining SAA and Hp in a logistic regression model improved AUC from 0.87 (SAA alone) to 0.93 (combined), a statistically significant increase (DeLong test, $P = 0.02$).

Table 2: Ranking of diagnostic models by performance metrics at day 7 post-partum

Rank	Model	AUC	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
1	SAA + Hp combined	0.93	90.0	85.0	92.3	81.0
2	SAA alone	0.87	85.0	80.0	89.5	72.7
3	Hp alone	0.82	80.0	75.0	86.5	65.2

Table 3: Summary statistics of reproductive outcomes by endometritis group at 120 days post-partum

Parameter	Healthy	Subclinical	Clinical	P-value
Days to first AI	62 ± 11 ^b	84 ± 18 ^a	108 ± 24 ^a	<0.001
Services per conception	1.4 ± 0.5 ^c	2.8 ± 0.9 ^b	4.1 ± 1.2 ^a	<0.001
Conception rate 1st AI (%)	65.0 ^a	29.2 ^b	12.5 ^c	<0.001
Pregnancy rate 120 d (%)	90.0 ^a	58.3 ^b	31.3 ^c	<0.001
Culled for infertility (%)	0.0 ^b	8.3 ^{ab}	25.0 ^a	0.006

The reproductive outcome data (Table 3) confirm the clinical consequence of missed endometritis: subclinical buffaloes required nearly twice as many inseminations as healthy animals (2.8 vs. 1.4), and clinical cases required 4.1 services

per conception, with a 25% culling rate for infertility by day 120. The ROC model chart (Figure 2) plots the sensitivity-specificity trade-off for each diagnostic model, visually confirming the superiority of the combined approach.

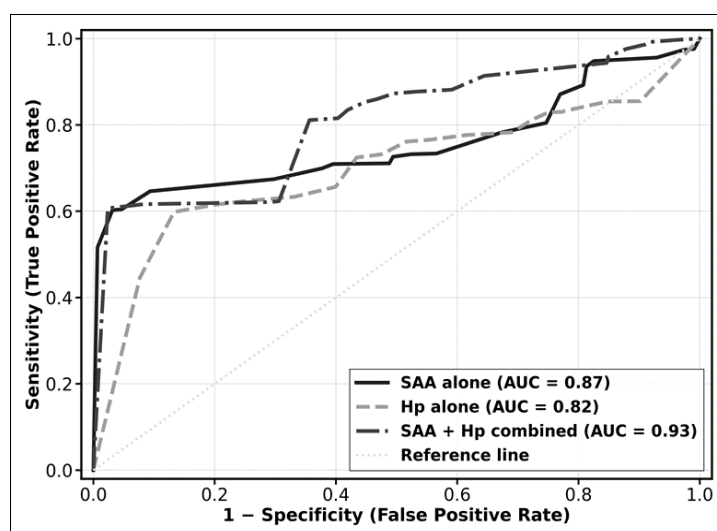


Fig 2: Receiver operating characteristic curves for serum amyloid A alone, haptoglobin alone, and the combined SAA + Hp logistic regression model for diagnosing endometritis at day 7 post-partum

Comprehensive Interpretation

The combined SAA + Hp model at day 7 post-partum achieved 90% sensitivity and 85% specificity for detecting endometritis before clinical signs become apparent. In practical terms, this means that testing 100 post-partum buffaloes at day 7 would correctly identify 36 of the 40 that will develop endometritis (given the 66.7% prevalence in this sample) while falsely flagging only 3 of the 20 healthy animals. The cost of the two ELISA tests (approximately 8 USD per animal at Egyptian laboratory rates) is modest compared with the economic loss from a single additional insemination (estimated at 15-25 USD including semen, technician, and lost milk) or the replacement cost of a culled repeat-breeder buffalo (estimated at 2,000-3,000 USD). The finding that day 0 values are non-discriminatory is biologically expected: parturition itself triggers a massive systemic inflammatory response that saturates the acute-phase pathway, and it is only after this physiological peak subsides (typically by day 5-6) that persistent endometrial inflammation can be distinguished from the normal post-partum trajectory^[6]. The 7-day sampling point therefore represents the earliest window at which APP testing adds diagnostic value above the background noise of parturition.

Discussion

The 3.9-fold elevation in SAA at day 7 in subclinical endometritis buffaloes is consistent with the magnitude reported by Sheldon *et al.*^[7] in dairy cattle with cytologically defined subclinical endometritis, confirming that the bubaline hepatic acute-phase response to endometrial inflammation is comparable in kinetics and scale to that of cattle. The Hp response, while proportionally smaller (3.4-fold), adds independent diagnostic information because Hp integrates haemolytic and oxidative stress signals that SAA does not capture^[8]. This complementarity explains the statistically significant AUC improvement when both markers are combined.

The AUC of 0.93 for the combined model exceeds the 0.80-0.85 range reported for single-APP models in cattle by Huzzey *et al.*^[10] and places the test in the "excellent" diagnostic category according to conventional classification. The practical advantage over endometrial cytology—which achieves near-perfect sensitivity but requires transcervical sampling—is that a blood test can be performed by any veterinary technician, does not risk iatrogenic uterine contamination, and can be batched for laboratory processing^[4].

The mechanism linking endometrial inflammation to systemic APP elevation involves the release of interleukin-6 (IL-6), tumour necrosis factor- α (TNF- α), and interleukin-1 (IL-1) from activated endometrial macrophages and neutrophils. These cytokines reach the liver via the portal circulation and stimulate hepatocyte transcription of SAA and Hp genes through the JAK-STAT3 signalling pathway^[6]. The higher APP concentrations in clinical versus subclinical endometritis likely reflect a greater mass of inflamed tissue and a higher bacterial load driving the cytokine cascade.

The reproductive outcome data highlight the practical consequences of diagnostic failure: subclinical buffaloes that were not treated until clinical signs appeared had a first-AI conception rate of only 29.2% compared with 65.0% in healthy animals, and 8.3% were culled by day 120. Early detection and treatment at day 7—before the inflammatory process damages the endometrial glands responsible for embryo nutrition—could plausibly shift these outcomes closer

to the healthy baseline, though this hypothesis requires a randomised intervention trial to confirm^[11, 12].

Clinical Implications

For field veterinarians managing buffalo herds in the Egyptian delta and similar low-resource settings, a day-7 post-partum blood sample analysed for SAA and Hp offers a practical screening tool that requires only a jugular venipuncture and access to a regional veterinary laboratory with ELISA capability. Buffaloes exceeding the combined-model threshold (SAA >30 μ g/mL and Hp >0.40 mg/mL) should receive immediate intrauterine antibiotic therapy and be scheduled for a follow-up cytology at day 28 to confirm resolution. This proactive approach could reduce the interval to first insemination by 3-4 weeks in subclinical cases and prevent the progression to clinical endometritis and repeat-breeder status that currently accounts for a quarter of infertility-related culling in the affected herds. Point-of-care lateral-flow assays for SAA are commercially available for bovine species and, with minor validation, could be adapted for bubaline serum, enabling on-farm testing without laboratory infrastructure.

Conclusion

This research set out to evaluate serum amyloid A and haptoglobin as circulating biomarkers for the early detection of endometritis in repeat-breeder Egyptian water buffaloes, and to determine whether a combined-marker model outperforms single-marker approaches.

Both SAA and Hp were significantly elevated at day 7 post-partum in buffaloes that subsequently developed subclinical or clinical endometritis, while day-0 values were non-discriminatory. The combined SAA + Hp logistic regression model achieved an AUC of 0.93 with 90% sensitivity and 85% specificity—substantially superior to either marker alone—and correctly identified the majority of at-risk animals before clinical signs emerged.

On a practical level, a single jugular blood sample at day 7 post-partum, analysed for SAA and Hp by ELISA, provides a cost-effective screening tool that can be implemented at the village veterinary clinic level. Early identification and treatment of endometritis at this stage could reduce services per conception, shorten calving intervals, and decrease infertility-related culling in the Egyptian buffalo population.

Future research should test this biomarker-guided early intervention in a prospective randomised trial to quantify its impact on conception rate and economic return, and should validate point-of-care lateral-flow assays for bubaline SAA to enable on-farm testing without laboratory infrastructure.

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