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Luteal dynamics and fertility response to post-insemination HCG and progesterone in sub-fertile buffaloes

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ABSTRACT

Luteal insufficiency, marked by inadequate progesterone (P_4) secretion, is a key cause of early embryonic mortality in buffaloes. The present study evaluated whether post-insemination administration of human chorionic gonadotropin (hCG) or P_4 could enhance luteal function and fertility in sub-fertile buffaloes. Thirty buffaloes free from subclinical endometritis and ovulatory defects were inseminated and assigned to three groups: control (saline), hCG (1500 IU on Days 4 and 11 post-AI), and P_4 (500 mg hydroxyprogesterone caproate on Days 4 and 11 post-AI). Ovarian structures and luteal blood flow were assessed using B-mode and Doppler ultrasonography, and pregnancy was diagnosed on day 55-60 post-AI. Conception rates were highest in the P_4 group (70%), followed by hCG (50%) and control (10%). Pre-ovulatory follicle size showed a positive correlation with CL area ($p<0.05$) and blood supply ($p>0.05$), but not with plasma P_4 concentration. CL area correlated significantly with blood supply ($p<0.05$), while plasma P_4 values on Days 4, 11, and 21 post-AI showed strong intra-day correlations. These findings indicated that post-AI supplementation with P_4 or hCG supported luteal development and functional activity and is associated with improved pregnancy outcomes in sub-fertile buffaloes, with the highest pregnancy rate observed following P_4 supplementation.

Keywords: Buffalo, Doppler, hCG, Progesterone, Sub-fertility

Buffalo is an important dairy animal in India, with a population of 109.85 million and contributing more than 50% of the total milk production (91.82 million tonnes out of 198.4 million tonnes) (20th Livestock Census, 2019). Owing to their adaptability, buffaloes can survive under hostile environmental conditions and play a significant role in ensuring nutritional security, strengthening rural livelihoods, and contributing substantially to the agricultural economy of the country (Balhara *et al.* 2022).

Despite their immense importance, reproductive inefficiency remains a major constraint to buffalo production, particularly in sub-fertile animals. These include poor estrus expression, silent estrus, seasonal and prolonged postpartum ovarian quiescence or anestrus, and embryonic mortality (Singh *et al.* 2001, Ghuman *et al.* 2010, Das and Khan, 2010, De Carvalho *et al.* 2016, Campanile *et al.* 2016). Embryonic mortality is particularly important during periods of increased photoperiod, with losses of 20–40% reported in artificially inseminated buffaloes and approximately 20% in naturally mated animals (Campanile and Neglia, 2007).

Luteal insufficiency and inadequate progesterone (P4) secretion are important contributors to embryonic mortality in buffaloes (Campanile *et al.* 2005; Saraswat and Purohit, 2016; Kumbhar *et al.* 2020). Compared with cattle, buffaloes possess fewer luteal cells within the corpus luteum (CL), contributing to lower circulating P4 concentrations (Baithalu *et al.* 2013). An adequate P4 during early embryonic development is essential for creating a receptive uterine environment and supporting maternal recognition of pregnancy through adequate embryonic interferon-tau secretion and prevention of luteolysis (Campanile *et al.* 2013, Lonergan *et al.* 2016).

The strategies to overcome luteal insufficiency aim to increase circulating P4 either by stimulating endogenous secretion or through exogenous supplementation. Post-AI administration of GnRH analogues or human chorionic gonadotropin (hCG) may stimulate CL activity and accessory CL formation, thereby increasing endogenous P4 secretion, whereas direct P4 supplementation provides exogenous luteal support during early embryonic development (Kumar *et al.* 2003, Pandey *et al.* 2013, Pandey *et al.* 2016b, Butani *et al.* 2016, Campanile *et al.*

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2007, Honparkhe *et al.* 2009, Ahmad and Arshad, 2020).

The hormone hCG, which is biologically similar to luteinizing hormone (LH), binds to LH receptors on small luteal cells and activates intracellular signalling pathways that enhance P4 synthesis (Stevenson *et al.* 2007). However, despite promising results with single post-AI treatments, information on the effects of repeated hCG or P4 administration in sub-fertile buffaloes is limited. Moreover, changes in luteal blood flow and functional activity of the CL following these treatments, as assessed by colour Doppler ultrasonography, remain largely unexplored in this category of animals.

Therefore, the present study hypothesized that administration of hCG or P_4 during the early and mid-luteal phases following AI would enhance luteal development and function and thereby improve fertility in sub-fertile buffaloes. Accordingly, the study aimed to evaluate the effects of hCG and P_4 administered on days 4 and 11 post-AI on corpus luteum development, luteal blood flow, plasma P_4 concentrations, and subsequent pregnancy outcome.

MATERIALS AND METHODS

A total of 30 healthy buffaloes, aged 5-8 years and of second to fourth parity, in good body condition and with a history of non-conception despite at least two inseminations with fertile semen (semen with post-thaw motility ≥50%), were included in the present study. The selected animals exhibited apparently normal cervico-vaginal mucus (CVM) discharge at estrus. The absence of subclinical endometritis at estrus was confirmed by polymorphonuclear cell (PMN) count in CVM, with values <5%. All experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC), vide letter no. GADVASU/2021/IAEC/62/08. The selected buffaloes (n=30) were artificially inseminated with frozen-thawed semen at the exhibition of overt estrus.

The study was conducted from October 2022 to April 2023, corresponding to the favourable breeding season for buffaloes in the region. All experimental buffaloes were maintained under uniform feeding and management conditions and were offered green fodder, dry fodder and concentrate mixture according to the standard farm feeding schedule, with free access to clean drinking water. Estrus detection was performed twice daily by three trained personnel during routine heat-detection parades, and animals exhibiting behavioural signs of estrus were

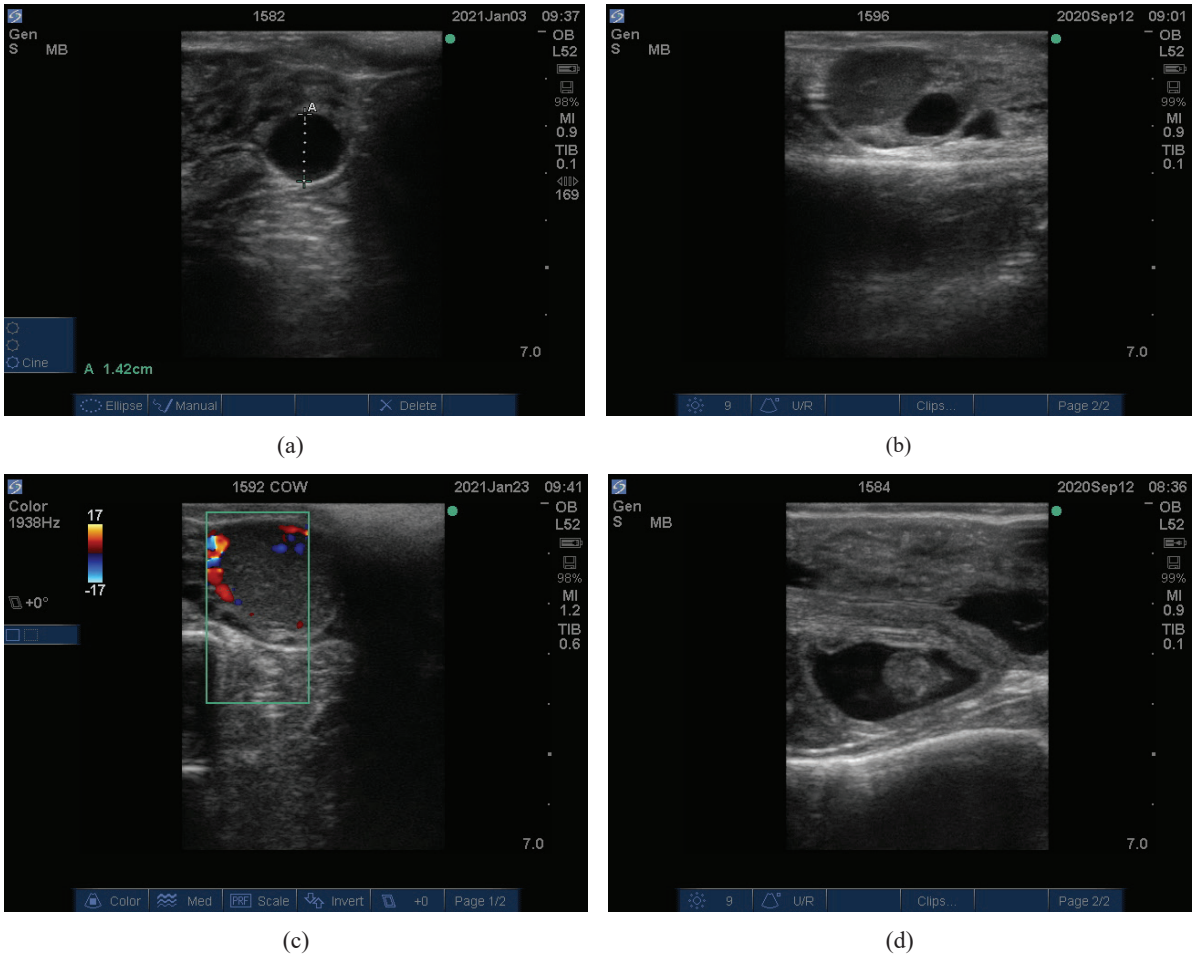

Fig. 1. Representative ultrasonographic images showing (a) pre-ovulatory follicle (POF) on the day of artificial insemination (AI) in B-mode; (b) follicle and corpus luteum (CL) on day 14 post-AI in B-mode; (c) colour Doppler image showing luteal blood flow on day 21 post-AI; and (d) ultrasonographic confirmation of pregnancy on day 47 post-AI.

subjected to further examination for confirmation. No estrus synchronization protocol was used in the study. Buffaloes with a history of failure to conceive following at least two previous inseminations were enrolled upon their subsequent spontaneous return to estrus and were inseminated at the observed estrus before allocation to the experimental groups.

Ovulation was confirmed 24h after artificial insemination (AI) with absence of dominant follicle, which was evident at the time of AI. Post-AI, the animals were randomly allocated into three groups based on treatment regimens. The Group I (hCG group; n=10) animals received 1500 IU hCG (Chorulon®, MSD) intramuscularly on days 4 and 11 post-AI. The animals in Group II (P4 group; n=10) received intramuscular injections of 500 mg hydroxyprogesterone caproate (Duraprogen®, Cargill) on days 4 and 11 post-AI. The animals of Group III (Control group; n=10) served as control and received intramuscular injections of 5 mL normal saline (placebo) on days 4 and 11 post-AI.

Transrectal ultrasonographic examinations of both ovaries were carried out on the day of AI, 24h after AI (to confirm ovulation) and subsequently on days 4, 11, and 21 post-AI using a portable ultrasound device equipped with a 5.0–7.5 MHz linear-array transducer (M-Turbo, Sonosite Inc., Bothell, USA). Pregnancy diagnosis was performed on days 55-60 post-AI. After evaluation of ovarian structures using B-mode ultrasonography, the color Doppler mode was activated to assess blood flow (Pixel^2) to the CL on days 4, 11, and 21 post-AI. Colored pixel aggregates indicating vascular signals were extracted and saved using Adobe Photoshop software (Adobe Photoshop CS3 Extended, Version 10.0, USA). Subsequently, ImageJ software (ImageJ 1.46, USA) was used to calculate the total number of colored pixels in each image, providing a quantitative measure of blood flow to ovarian structures.

Blood samples were collected five times from each buffalo, i.e. on the day of AI (day 0) and on days 4, 11, 21 and 50 post-AI, by jugular venipuncture into heparinized (1:1000) polystyrene tubes for estimation of plasma P_4 concentrations. Samples on days 0, 4, 11 and 21 post-AI were collected immediately before the corresponding ultrasonographic examinations. Plasma was separated by centrifugation (3000 rpm for 15 min) and stored at -20°C until estimation of P_4 concentration using an enzyme-linked immunosorbent assay (ELISA; DIA Source Immuno Assays SA).

Statistical analyses were performed using GraphPad Prism version 8.01 for Windows. Continuous data are presented as Mean ± SE, except where only a single observation was available. Pre-ovulatory follicle diameter among groups was analysed using one-way ANOVA. Longitudinal changes in plasma P_4 concentrations and luteal characteristics were analysed using repeated-measures ANOVA, with appropriate multiple-comparison procedures for comparisons across time and treatment groups. Pregnancy proportions among treatment groups were compared using an exact test appropriate for the small cell frequencies. Pearson's correlation coefficient was used to assess relationships among POF diameter, CL area, luteal blood flow and plasma P_4 concentrations. Differences were considered significant at $P<0.05$.

RESULTS AND DISCUSSION

The first service pregnancy rate (FSPR; %) was highest in the P_4 group (70%), followed by the hCG (50%) and control (10%) groups. The significantly higher pregnancy rate in the P_4 group than in the control group may be attributed to increased P_4 availability during the critical period of early embryonic development, thereby improving uterine receptivity and embryo survival. In contrast, hCG acts indirectly by stimulating luteal development and endogenous P_4 secretion. The numerically higher pregnancy rate following P4 than hCG treatment may therefore reflect the direct and sustained luteal support provided by exogenous P_4 supplementation.

The pregnancy rate observed following P_4 treatment was comparable to the findings of Awasthi *et al.* (2002) and Sharma *et al.* (2004), but higher than those reported following different P_4-based interventions by Patel *et al.* (2005), Sharma and Dhami (2008), Dhami *et al.* (2009), Pandey *et al.* (2013, 2016b), and Butani *et al.* (2016). Differences among studies may be related to the dose, formulation and timing of P_4 supplementation, animal category and reproductive status. The comparatively higher response in the present study may also be associated with repeated administration of 500 mg hydroxyprogesterone caproate on days 4 and 11 post-AI, although this interpretation requires confirmation in studies involving larger populations.

The pregnancy rate following hCG treatment was broadly consistent with those reported by Campanile *et al.* (2007), Honparkhe *et al.* (2009), Pandey *et al.* (2013, 2015), and Abo-Farw *et al.* (2020). Higher pregnancy rates reported by Pandey *et al.* (2016a, b) may be associated with differences in the timing of hCG administration, animal category and species. In particular, administration at AI may influence ovulation as well as subsequent luteal development, whereas the present study specifically evaluated post-ovulatory luteal support in sub-fertile buffaloes.

The mean pre-ovulatory follicle diameter did not differ significantly among the treatment groups on the day of AI ($p>0.05$), indicating a comparable follicular status before initiation of the treatments. The POF sizes recorded in the present study were comparatively smaller than those reported earlier, where larger POF were documented at AI (Honparkhe *et al.* 2009, Raj *et al.* 2018).

The mean POF diameter did not differ among groups on the day of AI ($p>0.05$), indicating comparable follicular status before initiation of post-AI treatments. Although age and parity may influence ovarian follicular dynamics, their specific associations with POF diameter were not evaluated in the present study; seasonal variation was also not assessed because the entire study was conducted

Table 1. Plasma progesterone (P4) concentrations (ng/mL) in relation to pregnancy status following post-insemination treatment in buffaloes

Days/ Pregnancy status	Group I (hCG; n=10)			Group II (P_4; n=10)			Group III (Control; n=10)		
	P (n=05)	NP (n=05)	Overall (n=10)	P (n=07)	NP (n=03)	Overall (n=10)	P (n=01)	NP (n=09)	Overall (n=10)
Day 0	0.44±0.04^{A}	0.60±0.03^{B}	0.52± 0.03^{a}	0.54±0.02	0.56±0.01	0.54± 0.01^{a}	0.56±0.0	0.57±0.05	0.57± 0.04^{a}
Day 4	1.69±0.02	1.71±0.01	1.70± 0.01^{b}	1.71±0.01	1.71±0.01	1.71± 0.01^{b}	1.78±0.0	1.78±0.01	1.78± 0.01^{b}
Day 11	3.26±0.24^{A}	2.34±0.22^{B}	2.80±0.22^{cC}	3.85±0.01^{A}	2.66±0.11^{B}	3.49±0.19^{cB}	2.41±0.0^{A}	1.93±0.08^{B}	1.98±0.09^{bA}
Day 21	3.95±0.17^{A}	0.87±0.17^{B}	2.41±0.53^{bc}	4.04±0.06^{A}	0.61±0.05^{B}	3.01±0.53^{cB}	3.46±0.0^{A}	0.88±0.15^{B}	1.14±0.29^{A}
Day 50	3.93±0.66^{A}	0.79±0.15^{B}	2.36±0.61^{bc}	4.78±0.14^{A}	1.05±0.08^{B}	3.66±0.58^{cB}	3.89±0.0^{A}	0.89±0.14^{B}	1.19±0.33^{A}

P: Pregnant; NP: Non-Pregnant

Values are Mean±SE. within each treatment group, means bearing different uppercase superscripts (A, B) within a row differ significantly (P<0.05) between pregnant and non-pregnant buffaloes. Means bearing different lowercase superscripts (a–c) within the Overall column differ significantly (P<0.05) among different days within the same treatment group. Means bearing different uppercase superscripts (A–C) within a row of the Overall columns differ significantly (P<0.05) among treatment groups on the same day.

during the favourable breeding season. Buffaloes that subsequently conceived had numerically larger POFs than non-pregnant buffaloes across all groups, although the differences were not significant (p>0.05). Similarly, Barile *et al.* (2010) reported larger POF diameters in buffaloes that became pregnant than in non-pregnant animals or those experiencing embryonic loss. In the present study, POF diameter was positively correlated with subsequent CL area (p<0.05) and luteal blood flow (p>0.05), but was not correlated with plasma P_4 concentrations on days 11 and 21 post-AI.

Plasma P_4 concentrations at different post-insemination intervals are presented in Table 1. Progesterone concentrations increased after AI in all groups, with comparatively higher values in P4-treated buffaloes than in the hCG and control groups during the post-insemination period. Similar increases in circulating P_4 during the luteal phase and following luteotrophic interventions have been reported in buffaloes (Carvalho *et al.* 2007, Honparkhe *et al.* 2009, Samir and Kandiel, 2019, Pandey *et al.* 2018, Abo-Farw *et al.* 2020). These findings indicated progressive luteal development after AI and supported the beneficial effects of post-insemination hormonal interventions on luteal function.

The increased P_4 availability during early embryonic development has been associated with improved embryo growth, viability and pregnancy establishment (Carter *et al.* 2008, Madureira *et al.* 2021). Similarly, hCG administration during early CL development can accelerate the post-AI rise in circulating P_4 concentrations (Maillo *et al.* 2014). Thus, the present findings supported the role of both exogenous P_4 supplementation and hCG-induced luteal stimulation in improving the P_4 environment required for early embryonic development and survival.

Plasma P_4 concentrations were significantly higher (P<0.05) in pregnant than in non-pregnant buffaloes on days 11, 21 and 50 post-AI across all three groups (Table 1). Similar differences in post-AI P_4 profiles between pregnant and non-pregnant buffaloes have been reported by Pandey *et al.* (2018) and Esposito *et al.* (2020), with higher P_4 concentrations during early luteal development being associated with subsequent pregnancy establishment.

Likewise, Gaur and Purohit (2020) observed higher P_4 concentrations during the early luteal phase in pregnant than in non-pregnant buffaloes, while Hassan *et al.* (2019) reported a marked decline in P_4 following luteal regression in non-pregnant animals. Lower luteal P_4 concentrations have also been reported in repeat-breeder than in normal cyclic animals (Honparkhe *et al.* 2006). Collectively, these findings supported the association of adequate luteal P4 secretion with pregnancy establishment, whereas insufficient luteal function might contribute to conception failure and embryonic loss.

On day 21 post-AI, pregnant buffaloes had significantly larger CL diameters (P<0.05) than their non-pregnant counterparts across all groups, with the greatest CL diameter observed in pregnant buffaloes of the P4 group (Table 2). Similar associations between greater CL development and pregnancy establishment have been reported in buffaloes. Esposito *et al.* (2020) observed larger CL areas in pregnant than in non-pregnant buffaloes during early luteal development, whereas Vecchio *et al.* (2012) demonstrated greater subsequent CL development in pregnant buffaloes following hormonal treatment. Daghash *et al.* (2022) further reported progressive CL growth in pregnant buffaloes, with differences from non-pregnant animals becoming evident during later stages of luteal development. Greater CL diameter has also been associated with improved luteal function and increased pregnancy probability (Velho *et al.* 2022). Collectively, these findings indicated that sustained CL development is closely associated with luteal competence and successful pregnancy establishment.

Luteal blood flow was higher in pregnant than in non-pregnant buffaloes, with significant differences (P<0.05) observed in the treatment groups at later post-AI assessment (Table 2). Similarly, Gaur and Purohit (2020) reported greater luteal blood flow in pregnant than in non-pregnant

Table 2. Corpus luteum diameter (mm) and luteal blood flow (Pixel²) in relation to pregnancy status following post-insemination treatment

Day	Parameter	Group I (hCG; n=10)		Group II (P_4; n=10)		Group III (Control; n=10)	
		P (n=05)	NP (n=05)	P (n=07)	NP (n=03)	P (n=01)	NP (n=09)
Day 4	CL diameter	9.85±0.55	9.2±0.65	11.57±0.42	10.67±0.34	10.17±0.16	11.00±0.57
	Blood flow	1920.1±508.4	1632.2±309.4	1827.6±102.62	1674.6±73.52	2315.2±0.0	1284.2±222.1
Day 11	CL diameter	11.71±0.90	10.00±0.42	13.57±0.29	10.00±0.76	12.17±0.16	10.56±1.15
	Blood flow	2006.4±266.2	1524.1±346.3	2062.5±207.2	1768.2±288.2	2564.1±0.0	1411.1±205.6
Day 21	CL diameter	13.71±0.36^{A}	7.60±0.16^{B}	14.43±0.48^{A}	7.66±0.16^{B}	14.17±0.16^{A}	7.80±0.46^{B}
	Blood flow	1865.3±535.8	638.2±343.5	2356.7±490.1^{A}	739.7±63.48^{B}	2676.2±0.0^{A}	903.2±287.8^{B}

P: Pregnant; NP: Non-Pregnant

Values are Mean ± SE. Within a treatment group, values bearing different superscripts (A, B) differ significantly (P<0.05) between pregnant and non-pregnant buffaloes on the same day.

Table 3. Correlation matrix between CL area, blood supply and progesterone values

Parameter D 4 PAI		CL area (mm²)			CL Blood pixel values			Plasma P4 con.		
		D 11 PAI	D 21 PAI	D 4 PAI	D 11 PAI	D 21 PAI	D 4 PAI	D 11 PAI	D 21 PAI	
CL area (mm²)	D 4 PAI	1.000								
	D 11 PAI	-0.176	1.000							
	D 21 PAI	0.064	0.494	1.000						
CL Blood pixel value	D 4 PAI	0.211	0.399*	0.031	1.000					
	D 11 PAI	0.389*	0.264	0.428*	0.180	1.000				
	D 21 PAI	0.225	0.116	0.025	0.046	0.202	1.000			
Plasma P4 con.	D 4 PAI	-0.080	0.028	-0.073	0.178	0.143	0.197	1.000		
	D 11 PAI	-0.095	0.253	0.351*	0.146	0.204	0.062	0.603*	1.000	
	D 21 PAI	-0.120	0.126	0.340*	0.030	0.161	0.370*	0.468*	0.814*	1.000

* indicates values are significant (p<0.05)

buffaloes during early luteal development, followed by a marked decline in non-pregnant animals with the onset of luteal regression. Greater luteal vascularity in pregnant buffaloes may reflect enhanced functional competence of the CL, as adequate blood perfusion facilitates substrate delivery for steroidogenesis and the transport of P_4 into circulation. Thus, assessment of luteal vascularity may complement morphological evaluation of the CL in determining its functional status during early pregnancy.

The correlation analysis revealed significant associations among selected indices of luteal development, vascularity and endocrine function (Table 3). CL area was positively correlated with luteal blood flow and plasma P4 concentrations at selected post-AI intervals. Plasma P4 concentrations also showed strong positive correlations across successive post-AI assessments, indicating that buffaloes with greater P4 concentrations during early luteal development tended to maintain higher concentrations during the subsequent luteal phase. Luteal blood flow was also positively associated with subsequent vascular and functional characteristics of the CL. These relationships indicated coordinated development of luteal structure, vascularization and steroidogenic function during the post-AI period.

The association among CL development, luteal vascularity and P4 secretion is biologically relevant because adequate vascular perfusion supports substrate delivery for steroidogenesis and facilitates hormonal exchange between the CL and systemic circulation. Velho *et al.* (2022) similarly reported positive associations among CL development, luteal blood perfusion, P4 secretion and pregnancy probability. Therefore, the combined assessment of CL morphology, luteal vascularity and circulating P4 may provide a more comprehensive indication of luteal competence than assessment of any single parameter alone.

In conclusion, post-AI administration of P_4 or hCG improved luteal function and fertility response in sub-fertile buffaloes, with the highest conception rate observed following P_4 supplementation. Greater CL development, luteal vascularity and circulating P_4 concentrations were associated with pregnancy establishment. Repeated P_4 supplementation on days 4 and 11 post-AI appears to be a promising approach for improving luteal support and fertility in sub-fertile buffaloes; however, further studies involving larger animal populations are warranted to confirm its reproductive benefits.

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REFERENCES

20th Livestock Census. 2019. All India report. Ministry of Agriculture, Department of Animal Husbandry, Dairying & Fisheries, Krishi Bhawan, New Delhi, India.

Abo-Farw M A E F, Elmalky O M, Elmaghraby E F, Elghait H A A G A and Aboul-Omran M A. 2020. An approach study for reducing the early embryonic loss in Egyptian buffalo-cows. *Physiology* **5**(2): 48–57.

Ahmad N and Arshad U. 2020. Synchronization and resynchronization strategies to improve fertility in dairy buffaloes. *Theriogenology* **150**: 173–179.

Awasthi M, Tiwari R and Mishra O P. 2002. Effect of progesterone supplementation during mid luteal phase on conception in repeat breeder crossbred cows. *Indian Journal of Animal Reproduction* **23**(1): 67–68.

Baithalu R K, Singh S K, Gupta C, Raja A K, Saxena A, Kumar Y and Agarwal S K. 2013. Cellular and functional characterization of buffalo (*Bubalus bubalis*) corpus luteum during the oestrous cycle and pregnancy. *Animal Reproduction Science* **140**(3–4): 138–146.

Balhara A K, Balhara S and Yadav P S. 2022. Buffaloes for nutritional secure and economically empowered rural India. *Advances in Animal Experimentation and Modeling*: 331–339. Academic Press.

Barile V L, Terzano G M, Allegrini S, Maschio M, Razzano M, Neglia G and Pacelli C. 2010. Relationship among preovulatory follicle, corpus luteum and progesterone in oestrus synchronized buffaloes. *Italian Journal of Animal Science* **6**(sup2): 663–666.

Butani M G, Dhami A J, Shah R G, Sarvaiya N P and Killedar A. 2016. Management of repeat breeding in buffaloes under field conditions using hormonal and antibacterial therapies. *Buffalo Bulletin* **35**(1): 83–92.

Campanile G and Neglia G. 2007. Embryonic mortality in buffalo cows. *Italian Journal of Animal Science* **6**(2): 119–129.

Campanile G, Di Palo R, Neglia G, Vecchio D, Gasparrini B, Prandi A, Galiero G and D'Occhio M J. 2007. Corpus luteum function and embryonic mortality in buffaloes treated with a GnRH agonist, hCG and progesterone. *Theriogenology* **67**(7): 1393–1398.

Campanile G, Neglia G and D'Occhio M J. 2016. Embryonic and foetal mortality in river buffalo (*Bubalus bubalis*). *Theriogenology* **86**(1): 207–213.

Campanile G, Neglia G, Gasparrini B, Galiero G, Prandi A, Di Palo R and Zicarelli L. 2005. Embryonic mortality in buffaloes synchronized and mated by AI during the seasonal decline in reproductive function. *Theriogenology* **63**(8): 2334–2340.

Campanile G, Vecchio D, Neglia G, Bella A, Prandi A, Senatore E M and Zicarelli L. 2013. Effect of season, late embryonic mortality and progesterone production on pregnancy rates in pluriparous buffaloes (*Bubalus bubalis*) after artificial insemination with sexed semen. *Theriogenology* **79**: 653–659.

Carter F, Forde N, Duffy P, Wade M, Fair T, Crowe M A and Lonergan P. 2008. Effect of increasing progesterone concentration from day 3 of pregnancy on subsequent embryo survival and development in beef heifers. *Reproduction, Fertility and Development* **20**(3): 368–375.

Carvalho N A T, Nagasaku E M, Vannucci F S, Toledo L M and Baruselli P S. 2007. Ovulation and conception rates according to intravaginal progesterone device and hCG or GnRH to induce ovulation in buffalo during the off breeding season. *Italian Journal of Animal Science* **6**(sup2): 646–648.

Daghash S M, Yasin N A, Abdelnaby E A, Emam I A, Tolba A and Abouelela Y S. 2022. Histological and hemodynamic characterization of corpus luteum throughout the luteal phase in pregnant and non-pregnant buffaloes in relation to nitric oxide levels based on its anatomical determination. *Frontiers in Veterinary Science* **9**: 1–12.

Das G K and Khan F A. 2010. Summer anoestrus in buffalo – a review. *Reproduction in Domestic Animals* **45**(6): e483–494.

De Carvalho N A T, Soares J G and Baruselli P S. 2016. Strategies to overcome seasonal anoestrus in water buffalo. *Theriogenology* **86**(1): 200–206.

Dhami A J, Butani M G and Sharma S K. 2009. Therapeutic management of repeat breeding buffaloes with hormones and antibiotics under field conditions. *Intas Polivet* **10**(1): 39–44.

Esposito L, Salzano A, Russo M, De Nicola D, Prandi A, Gasparrini B and Neglia G. 2020. Corpus luteum colour Doppler ultrasound and pregnancy outcome in buffalo during the transitional period. *Animals* **10**(7): 1181.

Gaur M and Purohit G N. 2020. Corpus luteum dimensions and vascularity reflect its functionality in pregnant and non-pregnant buffaloes. *Journal of Livestock Science* **11**: 101–105.

Ghuman S P S, Singh J, Honparkhe M, Dadarwal D, Dhaliwal G S and Jain A K. 2010. Induction of ovulation of ovulatory-size non-ovulatory follicles and initiation of ovarian cyclicity in summer anoestrous buffalo heifers (*Bubalus bubalis*) using melatonin implants. *Reproduction in Domestic Animals* **45**: 600–607.

Hassan M, Arshad U, Bilal M, Sattar A, Avais M, Bollwein H and Ahmad N. 2019. Luteal blood flow measured by Doppler ultrasonography during the first three weeks after artificial insemination in pregnant and non-pregnant *Bos indicus* dairy cows. *Journal of Reproduction and Development* **65**(1): 29–36.

Honparkhe M, Ghuman S P S, Dadarwal D, Jagir S and Dhaliwal G S. 2009. Embryonic mortality and luteal profile in buffaloes administered GnRH at the onset of oestrus or hCG 5 days after AI. *Indian Journal of Animal Sciences* **79**(8): 778–780.

Honparkhe M, Singh J, Dadarwal D, Kumar A and Gandotra V K. 2006. Luteal inadequacy as a possible cause of repeat breeding syndrome in dairy cattle. *Indian Journal of Animal Sciences* **76**(4): 296.

Kumar R, Phogat J B, Singh I, Kumar V, Singh U and Sethi R K. 2003. Efficacy of post-insemination progesterone supplementation for enhancement of fertility in buffaloes. *Bubalus Bubalis* **3**: 76–82.

Kumbhar U B, Deshpande V P, Gulavane S U and Gaikwad S M. 2020. Enhancement of conception rate through luteotropic hormones with special reference to metabolic, biochemical profile and cervical mucus properties in non-infectious repeat breeding Murrah buffaloes. *Buffalo Bulletin* **39**(1): 61–74.

Lonergan P, Forde N and Spencer T. 2016. Role of progesterone in embryo development in cattle. *Reproduction, Fertility and*

Development **28**(2): 66–74.

Madureira A M, Burnett T A, Borchardt S, Heuwieser W, Baes C F, Vasconcelos J L and Cerri R L. 2021. Plasma concentrations of progesterone in the preceding oestrous cycle are associated with the intensity of oestrus and fertility of Holstein cows. *PLoS One* **16**(8): e0248453.

Maillo V, Duffy P, O'Hara L, De Frutos C, Kelly A K, Lonergan P and Rizos D. 2014. Effect of hCG administration during corpus luteum establishment on subsequent corpus luteum development and circulating progesterone concentrations in beef heifers. *Reproduction, Fertility and Development* **26**(3): 367–374.

Pandey A K, Dhaliwal G S, Ghuman S P S and Agarwal S K. 2015. Impact of buserelin acetate or hCG administration on day 5 post-ovulation on subsequent luteal profile and conception rate in Murrah buffalo (*Bubalus bubalis*). *Animal Reproduction Science* **162**: 80–87.

Pandey A K, Ghuman S P S, Dhaliwal G S, Agarwal S K and Phogat J B. 2016a. Impact of buserelin acetate or hCG administration on the day of first artificial insemination on subsequent luteal profile and conception rate in Murrah buffalo (*Bubalus bubalis*). *Reproduction in Domestic Animals* **51**(4): 478–484.

Pandey A K, Ghuman S P S, Dhaliwal G S, Honparkhe M, Phogat J B and Kumar S. 2018. Effects of preovulatory follicle size on oestradiol concentrations, corpus luteum diameter, progesterone concentrations and subsequent pregnancy rate in buffalo cows (*Bubalus bubalis*). *Theriogenology* **107**: 57–62.

Pandey A K, Ghuman S P S, Dhaliwal G S, Kumar A and Agarwal S K. 2013. Impact of buserelin acetate or hCG administration on day 12 post-ovulation on subsequent luteal profile and conception rate in buffalo (*Bubalus bubalis*). *Animal Reproduction Science* **136**(4): 260–267.

Pandey N K J, Gupta H P, Prasad S and Sheetal S K. 2016b. Plasma progesterone profile and conception rate following exogenous supplementation of gonadotropin-releasing hormone, human chorionic gonadotropin and progesterone-releasing intra-vaginal device in repeat-breeder crossbred cows. *Veterinary World* **9**(6): 559.

Patel J A, Dhami A J, Kavani F S and Sarvaiya N P. 2005. Effect of hormonal therapies at breeding on plasma progesterone profile and fertility in repeat breeding Holstein Friesian cows. *International Journal of Cow Science* **1**(1): 52–59.

Raj M P, Naidu G V, Srinivas M, Raghunath M and Rao K A. 2018. Effect of preovulatory follicle on fertility in Graded Murrah buffaloes (*Bubalus bubalis*). *Indian Journal of Animal Research* **52**(6): 834–838.

Samir H and Kandiel M M. 2019. Accuracy of subjective evaluation of luteal blood flow by colour Doppler ultrasonography for early diagnosis of pregnancy in Egyptian buffalo. *Animal Reproduction Science* **208**: 106129.

Saraswat C S and Purohit G N. 2016. Repeat breeding: Incidence, risk factors and diagnosis in buffaloes. *Asian Pacific Journal of Reproduction* **5**(2): 87–95.

Sharma A, Jindal R and Singh R V. 2004. Progesterone supplementation on conception and biochemical profile in repeat breeding buffaloes. *Indian Veterinary Journal* **81**(9): 1036–1039.

Sharma S and Dhami A J. 2008. Effect of post-insemination antibiotics and hormone therapy on fertility in relation to macrominerals profile in repeat breeding animals. *Indian Journal of Field Vets* **3**(3): 1–6.

Singh B, Dixit V D, Singh P, Georgie G C and Dixit V P. 2001. Plasma inhibin levels in relation to steroids and gonadotrophins during oestrous cycle in buffalo. *Reproduction in Domestic Animals* **36**: 163–167.

Stevenson J S, Portaluppi M A, Tenhouse D E, Lloyd A, Eborn D R, Kacuba S and Dejarnette J M. 2007. Interventions after artificial insemination: Conception rates, pregnancy survival and ovarian responses to gonadotropin-releasing hormone, human chorionic gonadotropin and progesterone. *Journal of Dairy Science* **90**(1): 331–340.

Vecchio D, Neglia G, Gasparrini B, Russo M, Pacelli C, Prandi A and Campanile G. 2012. Corpus luteum development and function and relationship to pregnancy during the breeding season in the Mediterranean buffalo. *Theriogenology* **77**(9): 1811–1115.

Velho G D S, Rovani M T, Ferreira R, Gasperin B G and Dalto A G C. 2022. Blood perfusion and diameter of bovine corpus luteum as predictors of luteal function in early pregnancy. *Reproduction in Domestic Animals* **57**(3): 246–252.