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Corpus luteum development and function and relationship to pregnancy during the breeding season in the Mediterranean buffalo

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Abstract

The aim of this study was to ascertain corpus luteum (CL) development and function in buffaloes synchronized and mated by artificial insemination (AI) during the breeding season. Italian Mediterranean buffalo cows ($n = 43$) at 86.5 ± 2.7 days postpartum were synchronized by the Ovsynch-TAI Program and inseminated using frozen thawed semen at 20 and 44 h after the second injection of GnRH. The CL dimensions (diameter and area) and blood flow were examined on Days 5, 10, 15, 20, and 25 after AI by realtime B-mode/colour-Doppler ultrasonography. The resistive index (RI), pulsatility index (PI) and time average medium velocity (TAMV) were recorded at each time, together with CL dimensions. Blood samples were taken on the days of ultrasonography for progesterone (P4) assay by RIA. Data were grouped into pregnant or non-pregnant and retrospectively analyzed by repeated measure ANOVA and correlation analyses. Dimensions of the CL on Days 10, 20, and 25 after AI were greater ($P < 0.01$) in buffaloes pregnant on Day 45 ($n = 18$) compared with non-pregnant buffaloes ($n = 25$). The former buffaloes also showed a greater ($P < 0.01$) rate of CL growth between Days 5 and 10 after AI. Blood flow to the CL on Day 10 after AI showed a higher TAMV ($P < 0.01$) and lower RI ($P < 0.05$) in pregnant buffaloes compared with non-pregnant buffaloes. Negative correlations were observed on Day 10 after AI between CL diameter and RI ($r = -0.61$; $P < 0.01$) and PI ($r = -0.60$; $P < 0.01$); P4 concentrations and RI ($r = -0.46$; $P < 0.02$); and RI and pregnancy ($r = 0.45$; $P < 0.02$). Positive correlations were observed between pregnancy and CL size ($r = 0.54$; $P < 0.01$), ΔCL diameter between Days 5 and 10 ($r = 0.52$; $P < 0.01$), ΔCL area between Days 5 and 10 ($r = 0.48$; $P < 0.015$), and ΔP4 between Days 5 and 10 ($r = 0.50$; $P < 0.01$). Based on these findings it is concluded that the period between Day 5 and 10 is very important for CL growth and crucial in evaluating pregnancy. Accordingly, the assessment of CL parameters during the period from Day 5 to Day 10 after AI might be used to predict the likelihood of an ongoing pregnancy.

Keywords: Buffalo; Corpus luteum development; Color-Doppler ultrasonography; Pregnancy

1. Introduction

During the transition from long-day-length to short-day-length, function of the corpus luteum (CL) as judged by size and progesterone (P4) secretion can be

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reduced by 5% to 50% [1,2]. A typical scenario for buffaloes entering the transition period to seasonal anestrus is a progressive decline in CL activity in successive cycles before anestrus [2,3]. The mechanism whereby season influences CL function in buffaloes has undergone only preliminary investigation.

In a study conducted in transition buffaloes, the morphologic size of the CL determined by conventional ultrasonography was not closely related to P4 secretion or pregnancy to AI [4]. In the same study, CL vascularization and blood flow were associated with pregnancy [4]. Buffaloes with decreased CL vascularization had a greater CL blood resistance index (RI) on Day 25 after AI and reduced fetal growth [2]. Neglia et al. [5] reported a high RI in the preovulatory follicle (PF) of buffaloes that subsequently failed to conceive to AI. It would appear, therefore, that blood flow to both the PF and CL influences CL function and the pregnancy outcome to a synchronized AI in buffaloes. The above studies have demonstrated a basic underlying relationship between angiogenesis and function of the CL in buffaloes [2]. Studies in Holstein demonstrated that CL blood flow decreased approximately 2-fold from Days 15–17 (Day 0 = ovulation) and that it was closely correlated with a decrease in P4 secretion during the same period [6].

The present study examined the finer interrelationships between development and function of the CL and pregnancy in buffaloes synchronized during the peak seasonal breeding period. It was hypothesized that blood flow to the early developing CL is related to pregnancy outcome in buffaloes.

2. Materials and methods

2.1. Animals

The study was conducted in late autumn using 47 multiparous non-suckled Italian Mediterranean Buffalo cows at 87.3 ± 2.4 days in milk (40.5° N − 41.5° N parallel). The animals were selected from a larger group by clinical examination that included (1) rectal palpation of ovaries for follicular development (follicle ≥ 1.0 cm), (2) the presence of a corpus luteum to confirm cyclicity, and (3) the absence of gross abnormalities of the reproductive tract, such as uterine fluid. Animals were maintained in open yards that allowed 15 m^2 for each animal. A total mixed ration consisting of 50 to 55% forage and 45 to 50% concentrate, containing 0.90 milk forage units/kg of dry matter and 15% crude protein/dry matter, was fed daily in a group pen situation.

2.2. Estrus synchronization and AI

Stage of the estrous cycle was synchronized by using Ovsynch with timed-AI similar to that developed for cattle [7] and previously applied in buffaloes [8]. Briefly, a GnRH agonist (buserelin acetate, 12 μg; Receptal, Intervet) was administered on Day 0, a $PGF_{2\alpha}$ analogue (luprostiol, 15 mg; prosolvin, Intervet) on day 7, and GnRH agonist (12 μg) again on day 9. Cows were mated using AI by the same operator at 20 and 44 h after the second injection of GnRH agonist. Because of the relatively low intensity of estrous in buffaloes [9], animals were palpated per rectum (immediately before AI) to assess estrous status (follicle ≥ 1.0 cm and a tonic uterus with the presence or absence of mucous vaginal discharge).

2.3. Evaluation of corpus luteum development and function

Ovarian ultrasonography examinations were performed using a portable Sonoace Pico equipped with a 10 MHz linear transducer adapted for transrectal examination in large domestic animals. Features (dimensions and blood flow parameters) of the CL were examined on Days 5, 10, 15, 20, and 25 after AI. Once the ovary was visualized, the image was adjusted to give a better definition of the CL and then frozen for measuring the long and short axis, the color-Doppler mode was activated to display signals for blood flow in the CL and the spectral mode was applied to calculate the RI, pulsatile index (PI) and time average medium velocity (TAMV). All Doppler scans were performed at a constant color-gain setting, velocity setting, and a color-flow filter setting. The entire CL was scanned in a slow continuous motion. Real-time B-mode/colour-Doppler images of the continuous scans were recorded with a digital video-recording system for subsequent analysis.

2.4. Pregnancy

At the time of ultrasonography on Day 25 after AI, all buffaloes were also assessed for embryonic development. Pregnancies were confirmed on Days 45 and 70 after AI by rectal palpation. Buffaloes pregnant on Day 25 but not on Day 45 were considered to have undergone late embryonic mortality.

2.5. Progesterone

Function of the CL was evaluated by measuring circulating concentrations of P4 using RIA [10,11] in blood samples collected on Days 5, 10, 15, 20, and 25 after AI. Samples obtained from the jugular vein

Table 1
CL size, area and progesterone (P4) levels (ng/mL) in pregnant (P) and NP buffaloes on Day 5, 10, 15, 20, and 25 after artificial insemination (mean ± standard error).

Days	CL size (cm)		CL area (cm^2)		P4 (ng/mL)	
	P	NP	P	NP	P	NP
5	1.70 ± 0.00	1.70 ± 0.00	2.00 ± 0.20	2.30 ± 0.10	3.90 ± 0.30	3.60 ± 0.30
10	2.10 ± 0.00A	1.70 ± 0.00B	3.00 ± 0.20^{c}	2.40 ± 0.20^{d}	5.70 ± 0.30A	3.70 ± 0.30B
15	1.90 ± 0.00	1.70 ± 0.00	2.80 ± 0.10	2.40 ± 0.10	4.90 ± 0.30A	3.50 ± 0.30B
20	2.10 ± 0.00A	1.50 ± 0.00B	3.50 ± 0.20A	2.10 ± 0.10B	4.40 ± 0.30A	2.10 ± 0.30B
25	2.10 ± 0.00A	1.80 ± 0.00B	3.50 ± 0.10A	2.70 ± 0.20B	5.10 ± 0.30A	2.80 ± 0.30B

For each parameter values with different superscripts within rows are different ($^{cd}P < 0.05$; A, B, $P < 0.01$).

were centrifuged at 800 g for 15 min and the serum stored at −20 °C. P_4 concentrations >1.5 ng/ml were considered indicative of the presence of an active CL [12]. The minimum detectable amount of progesterone was 2.1 ± 0.08 pg and the intra- and interassay coefficients of variation were 6.2% and 11.8%, respectively.

2.6. Statistical analyses

Changes (Δ) in dimensions (diameter and area) of the CL and P4 concentrations were calculated for four periods after AI by subtracting the value on one observation with that of the previous observation: Period 1 P1, Day 5 to Day 10; Period 2 P2, Day 10 to Day 15; Period 3 P3, Day 15 to Day 20 and Period 4 P4, Day 20 to Day 25.

All data were tested for normality and no transformations were required. Differences between pregnant and non-pregnant buffaloes in CL dimension, CL growth, TAMV, RI and PI, and concentrations of P4 on Days 5, 10, 15, 20, and 25 after AI were analyzed by ANOVA SAS/and STAT [13]. Correlation analyses [13] were performed amongst CL dimension and growth (Δ), P4 concentrations, ΔP4, TAMV, RI and PI values, and pregnancy.

3. Results

A high proportion (43/47; 90%) of synchronized buffaloes responded to the Ovsynch protocol and were inseminated. The proportion of animals pregnant on Day 25 after AI was 19/43 (44.2%) and on Day 45 was 18/43 (41.9%). This finding indicated a low incidence (1/19; 5.3%) of late embryonic mortality.

The dimension of the CL on Days 10, 20, and 25 after AI was greater ($P < 0.01$) for buffaloes pregnant on Day 45 compared with non-pregnant buffaloes (Table 1). Buffaloes pregnant on Day 45 showed an increase in CL diameter (0.3 cm) and CL area (0.9 cm^2) from Day 5 to Day 10 (Period 1) after AI which did not occur in non-pregnant buffaloes (Table 2). The former buffaloes also showed an increase in CL area from Day 15 to Day 20 (Period 3) after AI while non-pregnant buffaloes showed a decrease in CL area during the same period (Table 2). Blood flow in the CL had a higher TAMV ($P < 0.01$) and lower RI ($P = 0.05$) in pregnant buffaloes compared with non-pregnant buffaloes on Day 10 after AI with no differences at other times (Table 3).

Buffaloes pregnant on Day 45 had greater ($P < 0.01$) concentrations of P4 on Days 10, 15, 20, and 25 after AI than non-pregnant buffaloes (Table 1). The

Table 2
CL growth (Δ) in terms of size and area, and progesterone (P4) levels (ng/mL) variations (∂) in pregnant (P) and NP buffaloes between the four periods considered (5–10 days after AI – P1, 10–15 days after AI – P2, 15–20 days after AI – P3, 20–25 days after AI – P4) (mean ± standard error).

Period	∂ CL size (cm)		∂ CL area (cm^2)		Δ P4 (ng/mL)	
	P	NP	P	NP	P	NP
P1	0.30 ± 0.10^{a}	0.00 ± 0.10^{b}	0.90 ± 0.20x	0.20 ± 0.20^{d}	1.80 ± 0.40A	0.03 ± 0.30B
P2	−0.20 ± 0.10	0.00 ± 0.10	−0.10 ± 0.20	−0.10 ± 0.20	−0.88 ± 0.41	−0.20 ± 0.40
P3	0.10 ± 0.10	−0.10 ± 0.10	0.60 ± 0.20A	−0.20 ± 0.20B	−0.53 ± 0.41	−1.50 ± 0.40
P4	0.10 ± 0.10	0.20 ± 0.10	0.10 ± 0.20	0.60 ± 0.30	0.69 ± 0.40	0.40 ± 0.40

For each parameter values with different superscripts within rows are different ($^{ab}P = 0.09$; x, $^{d}P = 0.06$; A, B, $P < 0.01$).

Table 3
TAMV, RI and PI in pregnant (P) and NP buffaloes on Day 5, 10, 15, 20, and 25 after artificial insemination (mean ± standard error).

Days	TAMV		RI		PI	
	P	NP	P	NP	P	NP
5	12.00 ± 1.50	11.70 ± 1.20	0.40 ± 0.00	0.40 ± 0.00	0.60 ± 0.10	0.50 ± 0.10
10	25.00 ± 1.30A	15.90 ± 1.40B	0.40 ± 0.00^{c}	0.50 ± 0.00^{d}	0.50 ± 0.10	0.80 ± 0.10
15	13.30 ± 1.20	13.40 ± 1.30	0.40 ± 0.00	0.40 ± 0.00	0.70 ± 0.10	0.70 ± 0.10
20	9.50 ± 1.20	10.10 ± 1.70	0.40 ± 0.00	0.40 ± 0.00	0.50 ± 0.10	0.60 ± 0.10
25	11.10 ± 1.20	13.10 ± 2.00	0.50 ± 0.00	0.40 ± 0.00	0.90 ± 0.10	0.60 ± 0.10

For each parameter values with different superscripts within rows are different ($^{cd}P < 0.05$; A, B, $P < 0.01$).

former buffaloes also had a greater positive ΔP4 from Day 5 to Day 10 (Period 1) after AI compared with non-pregnant buffaloes (Table 2). For both pregnant and non-pregnant buffaloes, the ΔP4 was negative from Day 10 to Day 15 (Period 2) and Day 15 to Day 20 (Period 3) after AI and positive from Day 20 to Day 25 (Period 4) after AI.

Negative correlations were found on Day 10 after AI between CL diameter and RI ($r = -0.607$; $P < 0.01$), CL diameter and PI ($r = -0.603$; $P < 0.01$), P4 concentrations and RI ($r = -0.462$; $P < 0.02$) and RI and pregnancy ($r = -0.450$; $P < 0.02$). Positive correlations were found between pregnancy and CL diameter ($r = 0.538$; $P < 0.01$), pregnancy ΔCL diameter ($r = 0.518$; $P < 0.01$), pregnancy and ΔCL area ($r = 0.479$; $P < 0.02$), and pregnancy and ΔP4 ($r = 0.499$; $P < 0.01$).

4. Discussion

The present study has demonstrated important differences in the development and function of the CL between pregnant and non-pregnant buffaloes. One of the key differences is thought to be the increase in both the diameter and area of the CL between Day 5 and Day 10 after AI in buffaloes that were pregnant on Day 45, which did not occur in non-pregnant buffaloes. Based on this observation it is suggested that the rate of CL growth between Day 5 and Day 10 could be a more accurate indicator of CL function, and predictor of the likelihood of pregnancy, than absolute size of the CL [14–16]. Also considered important was the increase in CL area between Day 15 and Day 20 in pregnant buffaloes and the decrease in CL area in non-pregnant buffaloes during the same period. The greater CL dimension in pregnant buffaloes was likely due, at least in part, to increased angiogenesis. In this regard, blood flow in the CL of pregnant buffaloes was characterized by a higher TAMV and lower RI on Day 10 after AI compared with non-pregnant buffaloes. Previous studies have shown a relationship between CL blood flow and pregnancy in buffaloes [4,5]. The importance of luteal blood flow was recently reported in cattle [17,18]. A 1.5-fold increase in luteal blood flow between Day 7 and 15 after estrus was observed in crossbred beef cows with pregnancy established by embryo transfer [17]. Dairy cows that conceived to AI showed greater blood flow to the CL during the third week after mating compared with cows that did not conceive [18].

The differences in CL dimension between pregnant and non-pregnant buffaloes were associated with differences in concentrations of P4. The greater positive ΔP4 from Day 5 to Day 10 after AI in buffaloes pregnant on Day 45 compared with non-pregnant buffaloes might have been of particular importance. In addition to this difference, pregnant buffaloes had greater P4 concentrations from Day 10 to Day 25 after AI. Progesterone supports the role of the uterus in embryonic development [19] and it is hypothesized that the greater CL dimension and increased P4 concentrations observed in pregnant buffaloes enhanced the function of the uterus, and increased the likelihood of pregnancy. This would be consistent with previous studies in buffaloes which have shown relationships between progesterone concentrations in the first two wks after AI and the fate of embryos [16,20,21].

In summary, the present study has provided important new information on the finer relationships between CL development and function during the first 2 wks after AI and pregnancy outcome in buffaloes. The design of the present study provides a model for future basic studies on the endocrinology of early pregnancy in buffaloes and there may also be potential for CL functionality to be developed into a predictor of pregnancy.

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