



Assessment of foetal lung surfactant in amniotic fluid of dogs by bubble test, to evaluate foetal maturity*

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Citation: Suprith, D.S., Unnikrishnan, M.P., Kurien, M.O., Bibin, B. B. and Binu, K. M. 2020. Assessment of foetal lung surfactant in amniotic fluid of dogs by bubble test, to evaluate foetal maturity. *J. Vet. Anim. Sci.* **51**(2): 128-131.

Received : 07.08.2019

Accepted : 15.10.2019

Published : 01-07-2020

Abstract

Study was conducted to determine the reliability of bubble test for assessment of lung surfactant in amniotic fluid and its application in clinical level to judge the foetal maturity. Amniotic fluid samples were collected from the fetuses of dogs undergoing elective caesarean section (Group I) at term and from the fetuses of dogs undergoing ovariohysterectomy at different stages of gestation, following misalliance (Group II). All the samples from neonates of Group I were found positive for bubble test, with variable grades ranging from two to four. All the samples from Group II fetuses were found negative for bubble test, with grade of zero and one. The results signify the presence of adequate foetal surfactant in amniotic fluid of mature fetuses when compared to that of immature fetuses and also suggest the potential practical utility of bubble test for foetal maturity assessment, particularly for timing elective induction of whelping or timing of caesarean delivery.

Keyword: Bubble test, Foetal maturity, Amniotic fluid, Surfactant, Gestation

Although 60 to 80 per cent of pregnant dogs suffering from dystocia end up in emergency caesarean section (Bergstrom *et al.*, 2006), risk to the dam and neonate is much higher (Moon *et al.*, 2000). Elective caesarean section (C-section) is an alternate approach but, with the risk of delivery of premature fetuses.

Rise in foetal adrenocorticosteroids and the decrease of progesterone concentration

*Forms part of the MVSc thesis submitted by the first author to the Kerala Veterinary and Animal Sciences University, Pookode, Wayanad, Kerala.

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during the final period of pregnancy are associated with the maturation of vital organs, including lung (Bonte *et al.*, 2017). Production of lung surfactant is the critical phase in the progression to lung maturity. It prevents atelectasis in neonates, by lowering alveolar surface tension, through formation of a phospholipid-rich monolayer between gas and liquid surfaces of alveolar epithelial cells. Impairment in the production of surfactants causes most significant pathological condition called respiratory distress syndrome (Vannucchi *et al.*, 2012).

Surfactant is produced by Type II pneumocytes during the saccular stage of lung development; *in-utero* foetal breathing movements and ciliated epithelium of the respiratory passages promotes diffusion of alveolar surfactant into amniotic fluid (Castagnetti *et al.*, 2007). This leads to the concept of sampling amniotic fluid for assessing foetal lung maturity. However studies for assessing foetal lung surfactant in dogs are lacking. Hence, the study was conducted to assess the presence of lung surfactant by bubble test, in the amniotic fluids collected during two different stages of pregnancy among dogs.

Materials and methods

The present investigation was carried out in University Veterinary Hospital, Kokkalai. A total of 12 dogs belonging to five different breeds were selected for the study and was divided in to two groups (Group I and II) of six each. Group I consisted of dogs which were undergoing elective C-section, when the serum progesterone level was < 2 ng/mL and Group II consisted of dogs which were undergoing ovariohysterectomy between 30 to 45 days of gestation following misalliance. About 1.5 to 3 mL each of amniotic fluid sample was aspirated from every foetal bag, into a 5 mL syringe, by needle puncture of amniotic sac between the foetal legs, before opening the foetal bags.

Amniotic fluid samples were analysed for the presence of lung surfactant, using bubble test as described by Bonte *et al.* (2017). Briefly, 1 mL of amniotic fluid sample was pipetted into a clean glass test tube and about 1 mL of 95 per cent of ethanol was added to it. Test tube was shaken vigorously for 30 sec and allowed to stand for 15 to 30 sec at room temperature and examined for the presence of bubbles at the meniscus. Grading of results was done by counting the number of layers of stable bubbles and inferred as depicted in Table 1.

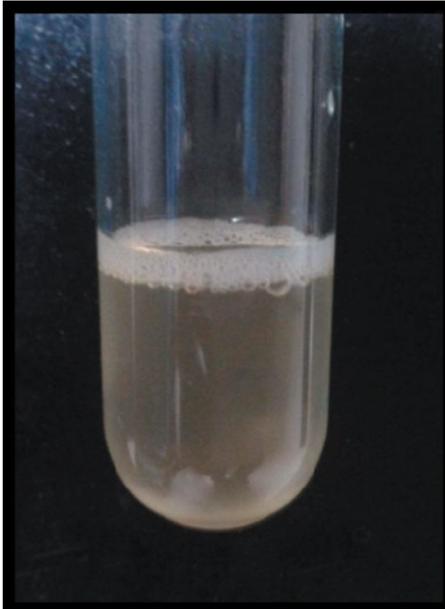
Table 1. Bubble test grading system used in the study

Observation	Grades	Inference
No bubbles	0	Negative
A single layer of bubbles	1+	
Two layers of bubbles	2+	Positive (Sufficient surfactant for <i>extra-uterine</i> life)
Three layers of bubbles	3+	
Whole surface of the tube covered with bubbles	4+	

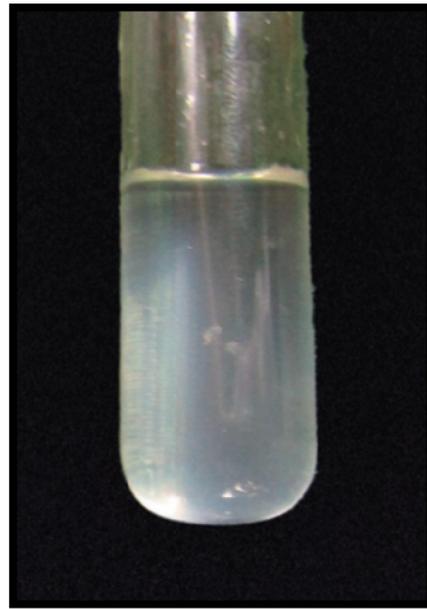
Table 2. Number and per cent of amniotic fluid samples showing different grades during bubble test

Group	No. of samples	Number and per cent of amniotic fluid samples with different bubble test grades				
		Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
I	24	0	0	11 (45.83%)	9 (37.5%)	4 (16.67%)
II	27	18 (66.67 %)	9 (33.33%)	0	0	0

Pictures showing positive and negative bubble test results in amniotic fluid



Positive bubble test result



Negative bubble test result

Results and Discussion

A total of 51 amniotic fluid samples were collected, among which 24 from Group I and 27 from Group II dogs.

All amniotic fluid samples of Group I were found positive for bubble test with variable grades ranging from two to four whereas all the samples from Group II were found negative for bubble test, with grade of zero and one. In Group I, 45.83, 37.5 and 16.67 per cent of samples showed bubble score of two, three and four, respectively. In Group II, 66.67 and 33.33 per cent of samples showed bubble score of zero and one, respectively.

The results indicate the presence of adequate lung surfactant in amniotic fluid to support *extra-uterine* life in Group I when compared to that of Group II. Jayakumar *et al.* (2018) also reported the positive results of bubble test in amniotic fluid collected from dogs undergoing C-section and negative results in amniotic fluid collected from those undergoing ovariectomy at different stages of gestation. Kutzler and Volkmann (2008) observed positive result of foam stability test in amniotic fluid collected at gestational age of

61 and 62 days, as calculated from Luteinizing Hormone (LH) surge and negative results in amniotic fluid collected at 59 and 60 days, past LH surge.

Negative results in amniotic fluids collected during ovariectomy at around 30 to 45 days of gestation in Group II foetuses could be attributed to fact that in canines, saccular stage of lung development was observed around 57 and 60 days of pregnancy and surfactant production might occur during that phase only (Sipriani *et al.*, 2009).

The results signify the presence of adequate foetal surfactant in amniotic fluid of mature foetuses when compared to that of immature ones and also suggest the potential practical utility of bubble test for foetal maturity assessment, particularly for timing elective induction of whelping or timing of elective C-section. Further studies are also essential to develop a harmless technique of collecting amniotic fluid from foetuses *in-utero*, like trans-abdominal amniocentesis and studies in large number of mature and premature puppies, which can find practical application in the management of canine pregnancy.

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