



# Changes in the Fetal Fluids Compositions during Dystocia of Dairy Buffaloes

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**Abstract** | Fetal fluids have different vital functions to sustain pregnancy and healthy parturition. Besides a deep understanding of fetal metabolism, diagnosis of pathological conditions during gestation and studying the composition of fetal fluids may provide information about fetal requirements for growth and maturation. With this background, the study was carried out in ninety pregnant buffaloes divided into two equal groups of eutocia and dystocia. Hormonal (cortisol and estradiol) and biochemical profile (cholesterol, glucose, total protein, creatinine, GGT, calcium, sodium, and potassium) of fetal fluid was measured for each of the buffalo. The cholesterol concentration in the case of dystocia had no significant difference from that found in the case of eutocia ( $P = 0.09$ ). The level of cortisol, glucose, creatinine, sodium, calcium, and GGT was significantly higher in buffaloes with dystocia ( $P < 0.05$ ). On the other hand, an increased estradiol concentration was observed in buffaloes with eutocia ( $P < 0.05$ ). Cholesterol and potassium concentration did not vary significantly in both groups of buffaloes ( $P > 0.05$ ). This study concluded that dystocia causes a significant change in hormonal and biochemical profiles of the fetal fluids. Hence, in addition to the fetus in the prenatal and postnatal stages of its life, an analysis of fetal fluids indicates the health status of the dam.

**Keywords** | Dystocia, Eutocia, Fetal fluids, Hormonal profile, Biochemical profile

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## INTRODUCTION

Fetal fluids, amniotic (AM), and allantoic (AL) have different vital functions that sustain pregnancy and normal parturition. One of the essential features is fetal fluid homeostasis. This is achieved by balancing the fluid volume and composition of the amniotic and allantoic compartments through an exchange between maternal and fetal circulations (Li et al., 2005).

Fetal fluids also play a significant role in physiologic exchanges between fetal and maternal tissues, therefore they are essential for the perfect handling of fetal waste products and prohibiting mechanical shock to the developing fetus during the whole pregnancy (Kamath-Rayne et al., 2014; Sheppard and Khalil, 2010; Vonnahme et al., 2015).

Amniotic fluid is vital to fetal health because it provides the fetus with a protective sac that prevents mechanical and thermal shock, possesses antimicrobial activity, helps in acid-base balance, and contains several nutritional factors (Stewart et al., 2001). Amniotic fluid also provides the fetus with amino acids (AAs), which are the building blocks for biomolecules included in physiologic growth and development, and precursors of nitrogenous substances with diverse regulatory functions (Wu, 2009).

Produced by the amnion epithelial cells, fetal tissues, fetal excretions, and placental tissues, a wide range of proteins and metabolites has been identified in amniotic fluid (Fanos et al., 2013; Shi et al., 2012).

These proteins can enter the amniotic fluid from the maternal uterine tissues, umbilical cord, amniotic fluid

cells, fetal urine, and other fetal secretions that include transudation through fetal skin (Jauniaux et al., 1998). Amniotic fluid proteins are principally of maternal origin. They have concentrations lower than maternal serum (Jauniaux et al., 1994) and, therefore, amniotic fluid reflects the physiological status during fetal development and it may be used to detect potential pathological conditions (Bazer et al., 2012; Pelizzo et al., 2014).

Any aberrations in the amniotic fluid could lead to fetal stress or diminish fetal development. Therefore, amniotic fluid provides the environment in which the fetus and its metabolic function develop. The volume and composition of amniotic fluid are consequently strictly regulated during development, reflecting the dynamic balance maintained through exchange between maternal circulation and fetal environment (Kwon et al., 2003; Wu et al., 1995; Baetz et al., 1975). On the other hand, allantoic fluid is composed of hypotonic urine, maintains osmotic pressure of the fetal plasma and prevents fluid loss to maternal circulation (Jainudeen and Hafez, 2000).

The concentration of components in amniotic and allantoic fluids is influenced by the exchange through the placenta, metabolic yields of the fetus, fetal urine formation, fluid-run through the urachus or urethra, and fetal secretions from lung and salivary glands. Nonetheless, the composition of amniotic and allantoic fluids differs substantially from that of fetal urine (Khajeh et al., 2007).

Studies on amniotic fluid composition are done for different animal species; however, they have been limited to only a few factors over a designated period of pregnancy. There exists a lack of data on differences in the hormonal and chemical composition of fetal fluid from eutocia to dystocia in buffaloes. This is the first study to elucidate the differences in the hormonal and biochemical balance between amniotic and allantoic fluids in buffaloes during eutocia and dystocia. Further to deep understanding of fetal metabolism and diagnosis of pathological conditions during gestation, studying the composition of fetal fluids may provide useful information about the requirements for the fetus growth, and maturation.

## MATERIALS AND METHODS

### ANIMALS

This study was carried out in ninety pregnant Egyptian nulliparous buffaloes (*Bubalus bubalis*) that were near the end of their gestation period (ranged between 312-320 days of gestation) and belonging to a private farm in Aswan province. Buffaloes were apparently healthy with no history of diseases with average age,  $28.7 \pm 0.9$  months. The buffaloes were divided into two equal groups of 45 each

(eutocia and dystocia). Buffaloes were fed green roughages (alfalfa and barley: clover mixture) *ad libitum* in addition to 4 kg/head/day of concentrate consisting of barley grains; 35%, wheat bran; 30%, maize; 17%, cotton; 10%, soybean meal; 5% as well as salt, vitamins, and minerals; 3%. Animals were vaccinated against brucellosis, foot and mouth disease, hemorrhagic septicemia.

Failure to progress in labor, either maternal and/or fetal causes, for instance, an obstruction or constriction of the birth passage or abnormal size, shape, position, or condition of the fetus, or pathological or difficult labor is referred to dystocia (Lombard et al., 2007; Zaborski et al., 2009; Uzamy et al., 2010).

### SAMPLING

Buffaloes which suffered from dystocia were undergoing the cesarean section. The gravid uteri were incised through greater curvature with a sharp scalpel to locate the fetal sacs, and then carefully separated from the endometrium and slowly enclosed outside the horn. The fetal fluids were collected by puncturing the amniotic and allantoic sacs. Samples of fetal fluids were collected from cases of dystocia (n=45) using a fine needle (30\_0.8 mm) and sterile (20-ml) syringes. In cases of eutocia (n=45) samples of fetal fluids were obtained transcervically during delivery. Samples were added in 20 mL sterile test tubes and stored at  $-20^{\circ}\text{C}$  for further analysis (Williams et al., 1992).

### BIOCHEMICAL ASPECTS DETERMINATION IN FETAL FLUIDS

Cortisol and estradiol-17 $\beta$  were assayed by radioimmunoassay using commercially available kits (Diagnostic Products Corporation, Los Angeles, USA) as per manufacturer's instruction. The assay had a sensitivity of 0.025 pg/dl and 8.0 pg/ml with an intra-assay coefficient of variations of 4.70 % and 5.30 %, respectively.

Cholesterol and glucose concentrations were quantitatively determined per the commercial kit's guidance (TECO diagnostic Company, Anaheim, CA, USA). Total protein (TP) was assessed using Biuret's method (Green et al., 1982). Creatinine (mg/dl) was measured by the kinetic method (Prestes et al., 2001). The sensitivity of the assay of creatinine was 0.31 mg/dL with linearity up to 20 mg/dL. Gamma-Glutamyl Transferase (GGT) was measured by the Kinetic colorimetric method (Szasz, 1976). The sensitivity of the assay of GGT was 2.0 U/L with linearity up to 600 U/L. Calcium (Ca), Sodium (Na) and Potassium (K) concentration were quantitatively determined as per manufacturer's instructions (Bio diagnostic Company). Milton Roy spectrophotometer (USA) was used to assess the absorbance for all of them. The sensitivity of the assay of Ca was 0.05 mmol/L with linearity up to 5 mmol/L, while in Na the assay is linear up to 250 mmol/L. In case of K, the assay is linear up to 10 mmol/L.

STATISTICAL ANALYSIS

The concentrations of mean±SD for concentrations of various biochemical components of fetal fluids were computed. To highlight the impact of eutocia and dystocia-associated variation in concentrations of various biochemical constituents of fetal fluids, the data was analyzed through student t-test. Tukey’s multiple comparison tests were conducted to test the significance between means. P<0.05, was considered significant.

RESULTS

The mean concentration of estradiol, cortisol, and cholesterol in amniotic fluid revealed a significant decrease in its levels as compared to concentrations in allantoic fluid from either normal parturition or dystocia (Tables 1 and 2). Cortisol concentration was significantly decreased in amniotic and allantoic fluids in buffaloes with eutocia than buffaloes with dystocia (Tables 3 and 4). Contrary to this, the level of estradiol in AF and AL had a significant increase in buffaloes with eutocia than buffaloes with dystocia (Tables 3 and 4). The concentration of cholesterol in both fluids in the case of eutocia has no significant difference from its level in buffaloes with dystocia (Tables 3 and 4). The mean glucose, total protein, creatinine, and GGT concentrations in amniotic fluid were significantly higher than their level in allantoic fluid in buffaloes of either normal parturition or dystocia (Tables 1 and 2). Except for total protein, a comparison for concentrations of glucose, creatinine, and GGT in AF and AL revealed an increased level in buffaloes with dystocia than eutocia (Tables 3 and 4).

Table 1: comparison of hormonal profile and biochemical characteristics between amniotic and allantoic fluids in buffaloes during normal parturition (eutocia).

Types of analysis	Analyzed parameter	Amniotic fluid (N=45)	Allantoic fluid (N=45)
Hormonal analysis	Cortisol (ng/ml)	1826±0.05*	2551±0.05
	Estradiol (pg/ml)	0.73± 0.08*	1.41± 0.03
Biochemical analysis	Cholesterol (mg/dl)	2 ± 0.04*	27±0.01
	Glucose (mg/dl)	75±0.07*	41±0.05
	Total protein(mg/dl)	3 ± 0.07	1 ± 0.04
	Creatinine (mg/dl)	12±0.03*	6.3±0.02
	GGT (U/L)	13±0.04*	8 ± 0.04
	Calcium (mmol/L)	1.35±0.05	1.87±0.06
	Sodium (mmol/L)	133.4±0.02	135.6±0.02
Potassium (mmol/L)	5.6 ± 0.02	5.3 ± 0.04	

The values are means ± SE; The values in each row with superscript differ significantly (P<0.05).

The mean Ca, and Na concentration in amniotic fluid were lower than its concentrations in allantoic fluid in the case

of normal parturition (Table 1), whereas their levels were decreased significantly in buffaloes with dystocia (Table 2). The concentration of Ca in AF and AL was considerably higher in buffaloes with eutocia than its level with dystocia (Tables 3 and 4). Similarly, the concentration of Na in AF and AL was significantly lower in buffaloes with eutocia than its level with dystocia (Tables 3 and 4). On the other hand, the average K concentrations did not vary significantly in the AF and AL of both groups (Tables 1, 2, 3 and 4).

Table 2: Comparison of hormonal profile and biochemical characteristics between amniotic and allantoic fluids in buffaloes during dystocia.

Types of analysis	Analyzed parameters	Amniotic fluid (N=45)	Allantoic fluid (N=45)
Hormonal analysis	Cortisol (ng/ml)	2001±0.03*	2815±0.07
	Estradiol (pg/ml)	0.21± 0.02*	0.75± 0.04
Biochemical analysis	Cholesterol (mg/dl)	2±0.06*	25 ± 0.05
	Glucose (mg/dl)	83±0.04*	55 ± 0.06
	Total protein(mg/dl)	2.1±0.04	0.8± 0.03
	Creatinine (mg/dl)	15±0.08*	7.5±0.02
	GGT (U/L)	20±0.07*	12 ± 0.05
	Calcium (mmol/L)	0.11±0.05*	0.75±0.01
	Sodium (mmol/L)	140±0.01*	145±0.05
Potassium (mmol/L)	5.1±0.08	5 ± 0.04	

The values are means ±SE; The values in each row with superscript differ significantly (P<0.05).

Table 3: Hormonal profile and biochemical characteristics of amniotic fluids in buffaloes during two different conditions of parturition (eutocia and dystocia).

Types of analysis	Analyzed parameters	Eutocia amniotic fluid	Dystocia amniotic fluid
Hormonal analysis	Cortisol (ng/ml)	1826±0.03*	2001±0.02
	Estradiol (pg/ml)	0.73± 0.02*	0.21± 0.05
Biochemical analysis	Cholesterol (mg/dl)	2 ± 0.02	2 ± 0.04
	Glucose (mg/dl)	75±0.06	83±0.09
	Total protein(mg/dl)	3 ± 0.08*	2.1±0.01
	Creatinine (mg/dl)	12±0.09	15±0.05
	GGT (U/L)	13±0.06*	20± 0.02
	Calcium (mmol/L)	1.35± 0.02*	0.11±0.08
	Sodium (mmol/L)	133.4±0.01*	140± 0.05
Potassium (mmol/L)	5.6 ± 0.09	5.1±0.01	

The values are means ±SE; The values in each row with superscript differ significantly (P<0.05).

DISCUSSION

Pregnancy in bovine species characterized by changes in the hormonal and biochemical constituents of fetal fluids, particularly during the last six months of gestation (Baetz et al., 1976).

**Table 4:** Hormonal profile and biochemical characteristics of allantoic fluids in buffaloes during two different conditions of parturition (eutocia and dystocia)

Types of analysis	Analyzed parameters	Eutocia allantoic fluid	Dystocia allantoic fluid
Hormonal Analysis	Cortisol (ng/ml)	2551±0.08*	2815±0.07
	Estradiol (pg/ml)	1.41± 0.07*	0.75± 0.04
Biochemical analysis	Cholesterol (mg/dL)	27± 0.07	25± 0.05
	Glucose (mg/dl)	41± 0.02	55± 0.05
	Total protein(mg/dl)	1 ± 0.04	0.8±0.09
	Creatinine (mg/dl)	6.3±0.05	7.5± 0.01
	GGT (U/L)	8 ± 0.07*	12± 0.02
	Calcium (mmol/L)	1.87± 0.03*	0.75±0.07
	Sodium (mmol/L)	135.6±0.07*	145± 0.03
	Potassium (mmol/L)	5.3 ± 0.04	5 ± 0.04

The values are means ±SE; The values in each row with superscript differ significantly (P<0.05).

Further to alteration in the relative contribution of the fetal and placental tissues to the amniotic and allantoic compartment, these changes indicate an occurrence of a change in metabolic and transport activity. Amniotic fluid surrounds the developing fetus and, therefore, the amniotic fluid composition and volume are affected by fetal urination, drinking as well as the permeability of fetal membranes (amnion and chorion). Furthermore, some hormones, such as cortisol and prolactin, may affect amniotic fluid composition indirectly by affecting the permeability of the fetal membrane (Wintour et al., 1986).

In the present study, the mean concentration of cortisol hormone in amniotic fluid revealed a significant decrease in both groups when compared to its concentrations in allantoic fluid. Contrary to this, a comparison between eutocia and dystocia revealed that the concentration of cortisol for either amniotic or allantoic fluid is significantly lower in eutocia than dystocia. These results are in agreement with the one reported previously by Bolis et al. (2017). They found that cortisol concentration was higher in the allantoic fluid than amniotic fluid (p<0.01) in canine species. Interestingly, Bolis et al. (2017) showed that cortisol has an important role in either survival or death of the neonate. They noticed a higher cortisol concentration in the amniotic fluid of puppies (p<0.05), that did not survive 24 hours after birth. Hence this particular parameter may be suggested for the neonate puppy's recognition, and, subsequently, special attention during the first day of age.

Accompanied by fetal lung maturity (Fencl and Tulchinsky, 1975), the concentration of cortisol in the amniotic fluid increases suddenly in the last seven days of gestation in humans (Divers et al., 1982). Though various sources produce unconjugated corticosteroids in amniotic fluid,

which are subjected to inter-conversion, De-Fencl et al. (2009) reported that the main source of corticosteroid conjugates is the fetal urine. Murphy et al. (1975) reported a strong correlation between cortisol of amniotic fluid and umbilical cord than that of maternal serum cortisol in human.

In the present study, cortisol concentration was higher in amniotic fluid in the case of dystocia than eutocia. Such differences may be attributed to the role of cortisol in parturition, especially in buffaloes with dystocia, which exerts high stress in the dam and the fetus at the same time.

Investigation of cortisol in the allantoic fluid has a lower clinical interest than amniotic fluid. It has been elucidated in some animal species such as the sheep (Challis et al., 1981) and tammar wallaby (Ingram et al., 1999). In tammar wallaby, with an average concentration of about 40 ng/ ml, the cortisol concentration in allantoic fluid increased toward the end of pregnancy. More recently, with concentrations of about 55 ng/ ml in feline species such as cat, Fresno et al. (2012) reported that cortisol concentration in allantoic fluid reached its maximum at the end term of pregnancy. A neonatal gender affects also been revealed for cortisol concentration in fetal fluids. For instance, Torday et al. (1981) reported higher amniotic concentrations of cortisol for females fetuses than males in humans.

Compared to estradiol concentration in allantoic fluid, the mean concentration of estradiol hormone in amniotic fluid revealed a significant decrease in both cases of eutocia and dystocia. Furthermore, a comparison between eutocia and dystocia revealed a significantly higher concentration of estrogen for either amniotic or allantoic fluid in eutocia than dystocia.

Parturition in most animals results from changes in circulating concentrations of hormone in the maternal and fetal circulations at the end of pregnancy. Robertson et al. (1985) carried out a study to correlate the concentrations of estrogens in the fetal fluids of the pig and the maternal blood and urine. The changes in the concentrations of estrone, oestradiol-17 beta, estrone sulfate, estradiol sulfates, and estrone glucuronide were assessed throughout pregnancy. At the end of the study, it was concluded that the pattern of change was similar for all estrogens measured in both fetal and maternal fluids suggesting an important role of this particular hormone in parturition.

The mean concentration of cholesterol in amniotic fluid revealed a significant decrease in its concentration compared to allantoic fluid either in normal parturition or dystocia. The concentration of cholesterol in amniotic fluid in eutocia had no significant difference than dystocia. These results differ from those reported previously

by [Abdulkareem et al. \(2012\)](#), who showed that the concentration of cholesterol in buffaloes was higher in amniotic fluid than allantoic during the 8<sup>th</sup> month of gestation. However, these differences may be attributed to the difference in time of cholesterol measurement since it was measured after the end of gestation. In this study, these results also are not in agreement with [Haffaf and Benallou \(2016\)](#), who showed that the cholesterol concentrations in fetal fluids were considerably higher than in later periods of gestation.

The mean glucose concentration in amniotic fluid was significantly higher in normal parturition and buffaloes with dystocia in this study, while it was decreased significantly in the allantoic fluid. These results are in agreement with [Haffaf and Benallou \(2016\)](#), who showed that, in the late pregnancy of sheep, the glucose level in the allantoic fluid becomes lower ( $P < 0.01$ ). Similar results were reported by [Kochhar et al. \(1997\)](#), who found a higher concentration of glucose in the amniotic fluid of equine species. Nonetheless, glucose concentrations might differ among species. For example, [Abdulkareem et al. \(2012\)](#) indicated a non-significant difference in glucose concentrations between the two types of fluids in buffaloes. [Murugavel et al. \(2014\)](#) observed a higher glucose concentration in the allantoic fluid than amniotic fluid in ovine. [Prestes et al. \(2001\)](#) found a significant difference in glucose concentration in ovine at the off end of pregnancy. [Barreto et al. \(2006\)](#) found a lack of difference between allantoic and amniotic fluid glucose concentration in the 40-day pregnant bitches.

The mean concentration of TP in amniotic fluid revealed a significant increase in both cases of eutocia and dystocia compared to allantoic fluid. The levels of TP in amniotic fluid in normal parturition were significantly increased than dystocia. These results are in agreement with [Abdulkareem et al. \(2012\)](#), who found a higher concentration of TP in the amniotic fluid of buffaloes than allantoic fluid. [Murugavel et al. \(2014\)](#) reported an increased level of TP in the amniotic fluid than the allantoic fluid in cattle species. In contrast, the concentration of allantoic and amniotic fluids in the present study significantly differed from those reported by [Kochhar et al. \(1997\)](#) in equine species. The TP concentrations in the amniotic fluid were similar to those reported by [Prestes et al., 2001](#) in the ovine during pregnancy. A low quantity of protein in fetal fluids of the present study was found similar to observations for other species ([Prestes et al., 2001](#); [Barreto et al., 2006](#)). [Reddy et al. \(1995\)](#) reported that a decreased concentration of total protein in amniotic fluid might be related to an absence of fibrinogen and other proteins due to fetal liver immaturity. The fetus uses dam-derived amino acids principally for synthesis rather than oxidation or gluconeogenesis ([Jainudeen and Hafez, 2000](#)). Thus, the perception of the average concentration of various biochemicals could be a

useful indicator in the determination of the physiological aspects of non-pregnant or pregnant animals. [Ocak et al. \(2014\)](#) revealed that the composition of amniotic fluid in mammals is affected by the excretion of fetal urine and that changes in the concentration of many components during late pregnancy may reflect the fetal metabolic activity. Furthermore, the TP deficiency in fetal fluids during parturition may be one of the causes that predispose the occurrence of dystocia

Mean creatinine concentrations in the amniotic and allantoic fluid were increased during normal parturition and dystocia. With the progression of pregnancy, the creatinine level increased in amniotic fluid, which indicates an increased protein metabolism in the fetus ([Anitha and Thangavel, 2011](#)). The conversion of creatinine in fetal muscle is the source of creatinine in amniotic fluid and, therefore, is a reflection of an increased fetal muscle mass and glomerular filtration ([Benzie et al., 1974](#)). Hence, glomerular filtration can be assessed by the concentrations of creatinine and urea in AF ([Ring et al., 1991](#)). In dystocia, the amniotic and allantoic fluid concentrations of creatinine were higher than eutocia. It has been proved that an increase in creatinine is correlated with kidney maturity ([Prestes et al., 2001](#)), and fetal muscular activity ([Kochhar et al., 1997](#)). An increased creatinine concentration has been reported in different abnormal conditions such as dystocia in bovine species and perinatal asphyxia in equine species. For instance, [Wendy \(1999\)](#), reported an increased creatinine concentration in equine fetuses that suffered from perinatal asphyxia syndrome due to decreased kidney perfusion blood flow.

The mean concentration of gamma-glutamyl transferase (GGT) in amniotic fluid revealed a significant increase in eutocia and dystocia compared to allantoic fluid in the same cases. In contrast, a comparison between eutocia and dystocia revealed that the concentrations of GGT for either amniotic or allantoic fluid are significantly lower in eutocia than dystocia. The GGT is a membrane-bound enzyme with emphasized functions in secretion and resorption and is being used for the diagnosis of liver diseases. Its activity is relatively high in livers of cows, horses, sheep, and goats. According to [Tainturier et al. \(1984\)](#), the activity of GGT enzymes shows an occasional irregular, small changes during pregnancy and early lactation.

The amniotic fluid showed a decrease in Ca concentrations compared to allantoic fluid during normal parturition and dystocia. However, while comparing both cases of parturition, eutocia had significantly higher levels than dystocia. These results were similar to those reported previously in equine species ([Fanos et al., 2013](#); [Kochhar et al., 1997](#)). Calcium is necessary for the activation of smooth muscle contraction during parturition. Therefore,

a low Ca levels in fetal fluid indicate its deficiency, which may be a cause of dystocia.

The average sodium ions concentration in the amniotic fluid did not show a significant decrease compared to allantoic fluid in both eutocia and dystocia. A comparison of the amniotic and allantoic fluid levels between eutocia and dystocia revealed a substantial decline in Na level in eutocia. These results were contrary to those observed previously in ovine species, (Prestes et al., 2001), who reported a higher concentration in the amniotic fluid than the allantoic fluid in all pregnancy phases.

The average potassium concentrations did not vary significantly in the amniotic and allantoic in eutocia and dystocia. These results differ from previous observation (Kochhar et al., 1997), who showed an increased concentration of K in equine species. They also indicated a higher K concentration in allantoic fluid than amniotic fluid. This may be attributed to an increase in the production of fetal urine as well as the increased fetal metabolic activity during pregnancy (Kochhar et al., 1997; Prestes et al., 2001).

## CONCLUSION

This study describes variations in the concentrations of cortisol, estrogen, and GGT in buffalo fetal fluids during two different conditions of parturition (eutocia and dystocia). Concluding the fact that fetal fluid monitoring may present details regarding potential cause of dystocia.

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## AUTHOR CONTRIBUTIONS

Dr. Yahia and Dr. Enas have made substantial contributions to the research design, or the acquisition, analysis or interpretation of data; and to drafting the manuscript while Dr. Eman revised it. All authors have approved the submitted version.

## CONFLICTS OF INTEREST

The authors have declared no conflict of interest.

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