



Gestational variations in the metabolites of the fetal amniotic fluid and maternal blood serum in Iraqi ewes at different stages of pregnancy

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Abstract

This study was conducted on 76 pregnant uteri were collected from 76 slaughter pregnant local Iraqi ewes (in different stages) in the Al-Shulla abattoirs/Baghdad province, the animals were intact in terms of health before slaughter and examined visually the genital tract after slaughter was natural and free from congenital malformations. This study performed from Dec. 2016 to Apr. 2017, their ages range from 3-5 years. The aspiration of amniotic fluid was performed by using sterile syringe. The content of amniotic fluid which kept in a test tube while the maternal blood serum performed by sterile syringe from jugular vein before slaughter animals and kept in test tube and the serum was isolated after 24 hours from blood collection in -5°C in refrigerator to determine the metabolites by using kits in spectrophotometer. The outcomes observed that the glucose was recorded significant differences ($p < 0.05$) in early pregnancy (amniotic fluid) & late pregnancy in maternal blood serum. But the total protein was significant highly ($p < 0.05$) in late pregnancy in amniotic & maternal blood serum, also urea and uric acid was recorded the same results which resembles of total protein. Finally the cholesterol was recorded non-significant in all stages of pregnancy in amniotic fluid but recorded highly significant ($p < 0.05$) in early pregnancy compared with late pregnancy in maternal blood serum. The outcomes recorded superior significant ($p < 0.05$ & $p < 0.01$) in all metabolites concentration related with maternal blood serum compared with amniotic fluid in different stages of pregnancy. In concluded that they recorded changes in metabolites related with different stages of pregnancy as well as the variations in concentration of metabolites were highly significantly in maternal blood serum compared with amniotic fluid and they considered one method of pregnancy diagnosis.

Keywords: Metabolites, Amniotic fluid, Maternal blood serum, Iraqi ewes.

التغيرات الأيضية للسائل السلي ومصل دم الام للنعاج العراقية الحوامل في مختلف مراحل الحمل.

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الخلاصة

انجزت الدراسة الحالية على 76 من ارحام النعاج الحوامل بعد ذبحها وفي مختلف فترات الحمل في مجزرة الشعلة – بغداد للفترة من كانون الاول 2016 لغاية نيسان 2017 وبأعمار تراوحت بين 3-6 سنوات. تم اخذ عينات من الدم عن طريق الوريد الوداجي لغرض الحصول على المصل الذي تم استخدامه في فحص الأيضات (Metabolites) لغرض مقارنتها مع ما موجود بالسائل السلي. سجلت نتائج البحث وجود فرق احصائي ($p < 0.05$) في الكلوكوز في المراحل المبكرة من الحمل بالنسبة لسائل السلي بينما الفرق الاحصائي سجل في المراحل المتأخرة من الحمل في مصل دم الام الحامل. اما البروتين الكلي فقد سجل

فارقاً احصائياً مهماً ($p < 0.05$) في المراحل النهائية للحمل في كل من السائل السلي ومصل دم الام الحامل وهذا ما تم تسجيله لكل من اليوريا وحامض اليوريك ايضاً. اخيراً ان الكولسترول لم يسجل اي فارق احصائي ($p < 0.05$) في جميع مراحل الحمل في السائل السلي مقارنة مع تسجيله فارقاً احصائياً مهماً ($p < 0.05$) في المراحل المبكرة مقارنة مع المراحل المتأخرة من الحمل في مصل دم الام الحامل. كما تشير نتائج الدراسة بان هنالك فارقاً احصائياً مهماً بمستوى ($p < 0.05$ & $p < 0.01$) في جميع تراكيز الايضات في مصل دم الام الحامل مقارنة مع السائل السلي في المراحل المختلفة من الحمل ويمكن اعتبار هذه الطريقة احدى طرق تشخيص الحمل. وعليه نستنتج من الدراسة بان التغيرات في تراكيز الايضات في كل من السائل السلي ومصل دم الام الحامل تسجل اختلافاً باختلاف مراحل الحمل وان تلك الاختلافات اكبر في مصل دم الام الحامل مقارنة مع السائل السلي.

الكلمات المفتاحية: الايضات, السائل السلي, مصل دم الام, النعاج العراقية.

Introduction:

Fetal membranes and fluid are matured for related with physiologic exchanges between fetus and tissues of dam and the fetal membranes are surrounded the embryo in nature (1 and 2). The system of feto-placental unite lead to constant exchange of water and fluids components between fetal fluid and circulation of dam, which is limited changes in physical, chemical and biochemical composition of fetal fluid (3 and 4). In cattle and sheep the amnion formation at 13-16 days of gestation, then the amniotic fluid and the amniotic sac (5 and 6). Amniotic fluid composed of large amount of proteins and metabolites produced by the amnion epithelial cell, fetal tissue, excretion of fetus and placental structures (7 and 8). Many authors reported that many significant differences between metabolites in different months of pregnancy (9, 10 and 11). Recent studies reported that the biochemical profiles both in maternal serum and fetal fluid is a tool for pregnancy diagnosis and state of growing fetus (12). The purpose of this study to determine the values of metabolites (glucose, total protein, urea, uric acid and cholesterol) in amniotic fluid and maternal blood serum at different stages of pregnancy in local Iraqi ewes.

Materials and methods

This study was performed on 76 pregnant uteri were collected from 76 slaughter pregnant local Iraqi ewes (in different stages) in the Al-Shulla abattoirs / Baghdad province, during period from Dec 2016 to April 2017 there ages are ranges from 3-6 years. The animals were intact in terms of health before slaughter and examined visually the genital tract

after slaughter was natural and free from congenital malformations. The aspiration of amniotic fluid was performed by using sterile syringe The content of amniotic fluid which kept in test tube within one week of collection while the maternal blood serum collected by sterile syringe from jugular vein before slaughter animals and kept in test tube and the serum was isolated after (24) hours from blood collection in -5°C in refrigerator determined the metabolites. analysis of metabolites in amniotic fluid & maternal blood serum by using special kits in spectrophotometer PD303-Germany. The statically analysis included mean, standard error, Chi-square and student test (F-test) according to (13).

Results and Discussion.

The outcomes observed in table- 1 that the glucose was recorded significant differences ($p < 0.05$) in early pregnancy related with amniotic fluid and these results were agreement with Khatun *et al.*, (4) and Mufti *et al.*, (14), but the significantly ($p < 0.05$) of late pregnancy in maternal blood serum (table -2), these findings agreement with McCrabb *et al.*, (15). But the total protein was significant highly ($p < 0.05$) in late pregnancy in amniotic and maternal blood serum, similar results were recorded by Khatun *et al.*, (4), also urea and uric acid was recorded the same results which resembled of total protein, these results which agree with Anitha and Thangavel, (16). Finally, the cholesterol was recorded non-significant differences ($p < 0.05$) in all stage of pregnancy in amniotic fluid (4 and 14), but recorded highly significant ($p < 0.05$) in early

pregnancy compared with late pregnancy in maternal blood serum(14).The outcomes recorded superior significant ($p<0.05$ and $p<0.01$) in all metabolites concentration related with maternal blood serum compared with amniotic fluid in different stage of pregnancy(table-3) and these results were agreement with many author's (17and 18) .We concluded from

this study that they differentiations in metabolites concentration in amniotic fluid and maternal blood serum related with stage of pregnancy and highly significantly ($p<0.01$) related with maternal compared with amniotic fluid and they considered one method of pregnancy diagnosis

Table -1: Metabolites concentration in amniotic fluid at different stages of pregnancy in local Iraqi ewes .

Metabolites	1 st month M±SE	2 nd month M±SE	3 rd month M±SE	4 th month M±SE	5 TH month M±SE
Glucose (mg/dl)	6.32±0.54a	6.13±0.39a	5.13±0.32a	3.14±0.54b	2.06± 0.26b
Total protein (mg/dl)	38.08± 2.13c	35.56± 2.43c	46.14± 2.13b	57.32±2.63a	61.14±3.22a
Urea (mg/dl)	25.14±3.56c	33.27±4.35c	38.25±5.43c	48.16±6.73b	62.56±4.36a
Uric acid (mg/dl)	0.71±0.2b	0.24±0.13c	0.27±0.03c	0.64±0.17b	0.94±0.32a
Cholesterol (mg/dl)	3.61±0.26a	3.84±0.38a	3.56±0.42a	4.01±0.28a	3.18±0.34a

Different letters mean sig .differences $p<0.05$

Table 2: Metabolites concentration in maternal serum at different stages of pregnancy in local Iraqi ewes.

Metabolites	1 st month M±SE	2 nd month M±SE	3 rd month M±SE	4 th month M±SE	5 TH month M±SE
Glucose mg/dl	48.14±1.57b	53.12±2.26b	51.32±2.35b	64.26±2.15a	67.10± 3.06a
Total protein mg/dl	46.13± 1.30d	42.22± 1.42d	54.24± 3.12c	67.25±3.74b	83.56±3.42a
Urea mg/dl	59.42±4.31d	78.56±4.56c	85.32±5.24c	93.42±3.74b	106.21±2.68a
Uric acid mg/dl	1.97±0.21c	2.08±0.24c	3.54±0.4b	5.56±0.37a	6.22±0.45a
Cholesterol mg/dl	112.54±7.19a	91.13±6.32b	83.25±4.82b	76.65±4.63c	62.14±3.86d

Different letters mean sig .differences $p<0.05$

Table - 3: Metabolites concentration in amniotic fluid & maternal serum at different stages of pregnancy in local Iraqi ewes.Different small letters mean sig. differences $p < 0.05$ within groupsDifferent capital letters mean sig. differences $p < 0.05$ & $p < 0.01$ between groups

Metabolites	Type of fluid	1 st month M±SE	2 nd month M±SE	3 rd month M±SE	4 th month M±SE	5 th month M±SE	Overall M±SE
Glucose mg/dl	Amniotic	6.32±0.54 aB	6.13±0.39 aB	5.13±0.23 aB	3.14±0.54 bB	2.06± 0.26bB	4.55± 0.41
	Maternal serum	48.14±1.5 7bA	53.12±2.2 6bA	51.32±2.3 5bA	64.26±2.1 5aA	67.10± 3.06aA	56.78± 2.27
Total protein mg/dl	Amniotic	38.08± 2.13cB	35.56± 2.43cB	46.14± 2.13bB	57.32±2.6 3aB	61.14±3.2 2aB	47.62± 2.50
	Maternal serum	46.13± 1.30dA	42.22± 1.42dA	54.24± 3.12cA	67.25±3.7 4bA	83.56±3.4 2aA	58.68± 2.6
Urea mg/dl	Amniotic	25.14±3.5 6cB	33.27±4.3 5cB	38.25±5.4 3CB	48.16±6.7 3bB	62.56±4.3 6aB	41.47± 4.88
	Maternal serum	59.42±4.3 1dA	78.56±4.5 6cA	85.32±5.2 4cA	93.42±3.7 4bA	106.21±2. 68aA	84.58± 4.10
Uric acid mg/dl	Amniotic	0.71±0.2b B	0.24±0.13 cB	0.27±0.03 cB	0.64±0.17 bB	0.94±0.32 aB	0.56±0. 17
	Maternal serum	1.97±0.21 cA	2.08±0.24 cA	3.54±0.4b A	5.56±0.37 aA	6.22±0.45 aA	3.87±1. 93
Cholesterol mg/dl	Amniotic	3.61±0.26 aB	3.84±0.38 aB	3.56±0.42 aB	4.01±0.28 aB	3.18±0.34 aB	3.64±0. 33
	Maternal serum	112.54±7. 19aA	91.13±6.3 2bA	83.25±4.8 2bA	76.65±4.6 3cA	62.14±3.8 6dA	85.14± 5.36

References:

1. Wahid, M. M; Hemeida, N. A; Shalash, M. R. and Isamil, E. M. (1991). Reproduction in Native Egyptian Cows: Faetal Membranes and Fluids. *Reprod. in Dom. Anim.*, 26(5), 270-273.
2. Prestes, N. C; Chalhoub, M. C. L; Lopes, M. D. and Takahira, R. K. (2001). Amniocentesis and biochemical evaluation of amniotic fluid in ewes at 70, 100 and 145 days of pregnancy. *Small Rumin. Res.*, 39(3), 277-281.
3. Faichney, G. J; Fawcett, A. A. and Boston, R. C. (2004). Water exchange between the pregnant ewe, the foetus and its amniotic and allantoic fluids. *J. of Comp. Physiol.* 174(6), 503-510.
4. Khatun, A; Wani, G. M; Bhat, J. I. A; Choudhury, A. R. and Khan, M. Z. (2011). Biochemical indices in sheep during different stages of pregnancy. *Asian J. Anim. Vet. Adv.*, 6(2), 175-181.
5. Robert, S.Y. and Walter, R.T. (2007). Current Therapy in the Large Animal, Theriogenology 2nd Ed. Saunders- Elsevier.
6. Noakes, D. E; Parkinson, T. J. and England, G. C. (2009).

- Veterinary Reproduction and Obstetrics. London: Sounders Elsevier, pp. 751-776.
7. Shi, L; Mao, C; Zeng, F; Zhang, L. and Xu, Z. (2012). Central angiotensin I increases swallowing activity and oxytocin release in the near-term ovine fetus. *Neuroendocrinology*, 95(3), 248-256.
 8. Fanos, V; Atzori, L; Makarenko, K; Melis, G. B. and Ferrazzi, E. (2013). Metabolomics application in maternal-fetal medicine. *Biomed. Res. Intern.* 2013:720514.
 9. Olfati, A; Moghaddam, G; Kor, N. M. and Bakhtiari, M. (2014). The relationship between progesterone and biochemical constituents of amniotic fluid with placenta traits in Iranian crossbred ewes (Arkhar-Merino× Ghezel). *Asi. Pacif. J. of Trop. Biomed.*, 7, S162-S166.
 10. Haffaf, S. and Benallou, B. (2016). Changes in energetic profile of pregnant ewes in relation with the composition of the fetal fluids. *Asi. Pacif. J. of Trop. Biomed.*, 6(3), 256-258.
 11. Busardo, F.P; Frati, Zaami, S. and Fineschi, V. (2015). Amniotic fluid Embolism Pathophysiology Suggests New Diagnostic Armamentarium : B-Tryptase And Compleat Fractions C3-C4 Are The Indispensable Working Tools. *Int.J.Mol.Sci*:16, 655-657.
 12. Tabatabaei, S. (2012). Gestational variations in the biochemical composition of the fetal fluids and maternal blood serum in goat. *Comp .Clin .Path.*, 21(6), 1305-1312.
 13. Steel, R. G and Torrie, H. J. (1986). Principles and procedures of statistics. 2 edition, Mc Graw Hill. London .
 14. Mufti, A. M. (1995). Studies on morphological characters and some metabolic constituents at different stages of development of ovine foetus. MV Sc (Doctoral dissertation, Thesis, Sher-e-kashmir University of Agricultural Sciences and Technology).
 15. McCrabb, G. J; Egan, A. R. and Hosking, B. J. (1991). Maternal under nutrition during mid-pregnancy in sheep. Placental size and its relationship to calcium transfer during late pregnancy. *Brit. J. of Nut.*, 65(02), 157-168.
 16. Anitha, A. and Thangavel, A. (2011). Biochemical profile of ovine amniotic and allantoic fluids. *Tamilnadu J. Vet. & Anim. Sci*, 7, 262-267.
 17. Bazer, F. W; Spencer, T. E. and Thatcher, W. W. (2012). Growth and development of the ovine conceptus. *J. of anim. Sci.*, 90(1), 159-170.
 18. Aidasani, R; Chauhan, R. A. S; Tiwari, S. and Shukla, S. P. (1992). Some metabolic constituents of caprine fetal fluids and fetal serum. *Ind. J. of Anim.Sci.*, 62(4), 335-336.