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Changes in energetic profile of pregnant ewes in relation with the composition of the fetal fluids

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ABSTRACT

Objective: To evaluate the energetic profile of fetal fluids and to make comparisons of the concentrations of the constituents present with those in the maternal plasma.
Methods: A study was conducted in 102 gravid sheep uteri. The four stages of gestation as Stage I (0–60 days), Stage II (61–90 days), Stage III (91–120 days) and Stage IV (121–145 days) were identified based on the crown anus length of the embryo/fetus. The amniotic and allantoic fluids collected from the gravid uteri of each group were subjected to biochemical analysis of glucose, cholesterol and triglyceride.
Results: The levels of glucose and triglyceride in maternal plasma were lower ($P < 0.05$) on late pregnancy as well as in amniotic and allantoic fluids. No significant variation ($P > 0.05$) of plasma cholesterol levels was detected between the sampling periods. Contrariwise, cholesterol concentrations of fetal fluids were higher in Stages III and IV of pregnancy when compared with the Stages I and II.
Conclusions: The influence of pregnancy on the biochemical composition of fetal fluids was statistically significant.

1. Introduction

During pregnancy, the growing fetus principally takes nutrient supplies from the mother depending on uterine blood flow. Indeed, fetal and placental weights and uterine blood flow are highly correlative [1,2]. Fetal fluids are important for physiologic exchanges between fetal and maternal tissues, so they are necessary for the efficient handling of fetal waste products and preventing mechanical shock to the developing fetus during entire gestation [3–5].

A high relative rate of growth demands a highly specific pattern of substrate and metabolic intermediates for ordered development of cells, tissues and organs [6]. Thus, the quality of fetal nutrition is most important early in gestation [6,7].

The concentration of components in amniotic and allantoic compartments is affected by the exchange through the placenta, metabolic yields of the fetus, fetal urine formation and fluid run through the urachus or urethra and fetal secretions from lung and salivary glands. However, amniotic and allantoic fluids differ substantially in composition than that of fetal urine [8]. Amniotic fluid composition reflects the physiological status during fetal development and it may be used to detect potential pathological conditions [9,10]. Amniotic fluid contains large amounts of proteins and metabolites produced by the amnion epithelial cells, fetal tissues, fetal excretions and placental tissues [11,12].

Studying the composition of maternal plasma and fetal fluids provides useful information about the requirements for fetus, fetal growth and maturation. It is observed that several circulatory and transport properties of the ewe's placenta are as the same as those of the human placenta and as such, for that, the pregnant sheep helps greatly in giving an outstanding model to study the growth of fetus [13].

Glucose is indispensable for the fetus development since it is considered mainly as a source of energy. The sheep fetus uses this substance as a chief metabolite and because of the speedy development of the fetus, the energy necessities of the ewe augment throughout late pregnancy [14].

Studies on sheep also paid much attention to the change that occurs in the composition of fetal fluid in early pregnancy [15]. This change is a reflection to changing metabolic and transport activity as well as modification in the relative involvement of the fetal and placental tissues to the amniotic and allantoic compartments [16].

In understanding fetal metabolism and identifying pathological conditions throughout pregnancy, a broad knowledge of amniotic fluid is required [17].

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The current investigation was conducted to evaluate the energetic profile of the fetal fluids and maternal plasma throughout different stages of pregnancy in ewes.

2. Materials and methods

A total of 102 female genitalia from pregnant ewes were collected aimlessly from local slaughterhouse. The uteri were immediately collected after slaughter, washed and brought to the laboratory in ice. Uteri were opened along the dorsal curvatures and maternal caruncles were separated gently from fetal cotyledons. The intact amnion and allantois along with embryo or fetus were separated. Allantoic and amniotic sacs were punctured and 10 mL of allantoic and amniotic fluids were aspirated from each sac. About 10 mL sterile syringes and needles were used for collection of these fluids. Samples were stored in labeled plastic tubes and frozen at −20 °C until biochemical analysis. The samples of jugular blood were gathered from ewes in sterilized glass tubes. In an icebox, these tubes were put and taken to the laboratory. These samples were dealt with under specific conditions and they were centrifuged at 4 000 r/min for 3 min. On the other hand, the plasma was separated and stored at −20 °C for further analysis. The embryo/fetuses were removed from the uterus and the crown anus length of the fetus from the vertex of skull to the anus was measured using a measuring tape. The stages of gestation and age of the fetus were determined by applying the Keller formula: $L = X\ (X + 3.5)$ [18], where X denotes the developmental age in months and L is the crown-rump length (cm). Based on the age of the fetus, the course of gestation was divided into four stages as: Stage I (0–60 days), Stage II (61–90 days), Stage III (91–120 days) and Stage IV (121–145 days). The samples of plasma and fetal fluid were made under analysis for some biochemical metabolites (glucose, cholesterol and triglyceride) using commercially available kits by photometer method on an autoanalyzer Selectra junior.

The values of mean ± SD for concentrations of various biochemical components of fetal fluids and plasma were computed. To highlight the impact of gestational age variation in concentrations of various biochemical constituents of fetal fluids and plasma, the data were directed to One-way ANOVA. Tukey's multiple comparison test was conducted to test significance between means. If $P < 0.05$, it was tended to consider the differences as significant.

3. Results

The mean values and SD for some biochemical metabolites levels of fetal fluids and the maternal plasma at different stages of gestation in ewes were presented in Table 1.

There was a marked decrease in the levels of glucose and triglyceride in maternal plasma with advancement of pregnancy as well as in amniotic and allantoic fluids. No significant variation ($P > 0.05$) of plasma cholesterol levels was detected between the sampling periods. Contrariwise, cholesterol concentrations of fetal fluids were higher in Stages III and IV of pregnancy in comparison with the Stages I and II.

4. Discussion

In this study, it is observed that there is a significant decrease of glucose concentration in advanced pregnancy in fetal fluids. These results were mainly in agreement with former results in ewes and cattle [19–21], but they were different from those reported in sheep [22]. In late pregnancy, the glucose level in the allantoic fluid was notably lower ($P < 0.01$) and this finding is different from other reports [23]. During all the probation period, the level of glucose in maternal plasma was significantly higher than that of fetal fluids. Indeed, with advancing pregnancy, the glucose values in maternal plasma were decreased. This result was mainly in agreement with those reported in sheep [24–26]. They concluded that fetal intake of glucose due to the development of the fetal swallowing reflex may be the cause behind decreasing glucose levels in gestation.

There were no significant differences of cholesterol concentration in maternal plasma between the sampling periods. Many studies reported that with advancing pregnancy in ewes, a decreasing trend of the serum cholesterol level was marked. During all stages of pregnancy, the cholesterol level in maternal plasma was significantly higher in comparison to that of fetal fluids.

In the late stages of gestation, the cholesterol concentrations in fetal fluids were considerably higher than in earlier gestation. The marked increase in cholesterol level with advancement of pregnancy in amniotic and allantoic fluids meets the need for the synthesis of progesterone [27].

With advancing pregnancy, there was a slow decrease in the levels of triglycerides in fetal fluids and maternal plasma. This finding is similar to results in goats [28]. Triglycerides are the store of lipids of animals in the plasma where the body uses them, mainly as fuel. When energy necessities increase, mainly in advanced pregnancy, the ewes go to consume the plasma lipids [29].

To conclude with, the concentrations of glucose, cholesterol and triglycerides in fetal fluids and maternal plasma of pregnant ewes were changed with advancing gestation stages. Based upon the evaluation of energetic profile, it may be possible to detect the early aberrations in metabolism and thereby, appropriate

Table 1

Levels of metabolite constituents of fetal fluids and maternal plasma during different stages of pregnancy in ewes.

Gestation days	Glucose (g/L)			Cholesterol (mg/dL)			Triglyceride (mg/dL)		
	Maternal plasma	Amniotic fluid	Allantoic fluid	Maternal plasma	Amniotic fluid	Allantoic fluid	Maternal plasma	Amniotic fluid	Allantoic fluid
0–60 (n = 34)	0.62 ± 0.15^{a}	0.24 ± 0.08^{a}	0.21 ± 0.07^{a}	87.65 ± 20.31	2.65 ± 1.75^{abc}	1.79 ± 1.44^{abc}	28.65 ± 9.80^{ab}	14.91 ± 4.54^{ab}	21.71 ± 7.50^{ab}
61–90 (n = 25)	0.46 ± 0.12^{bcd}	0.14 ± 0.05^{bc}	0.11 ± 0.03^{bc}	82.20 ± 17.39	2.84 ± 1.30^{abc}	2.01 ± 1.17^{abc}	25.88 ± 7.90^{ab}	12.72 ± 4.97^{ab}	18.56 ± 7.74^{ab}
91–120 (n = 23)	0.41 ± 0.12^{bcd}	0.09 ± 0.03^{bcd}	0.08 ± 0.03^{bcd}	78.04 ± 15.35	3.52 ± 1.72^{abc}	2.40 ± 1.25^{abc}	17.17 ± 3.70^{cd}	9.34 ± 2.38^{cd}	12.70 ± 3.19^{cd}
121–145 (n = 20)	0.39 ± 0.12^{bcd}	0.06 ± 0.03^{cd}	0.04 ± 0.02^{cd}	77.50 ± 13.62	4.35 ± 1.59^{cd}	3.10 ± 1.54^{cd}	15.60 ± 5.10^{cd}	9.25 ± 4.01^{cd}	10.65 ± 3.55^{cd}

^{a,b,c,d}: The values in each column with different letters were significantly different ($P < 0.05$).

corrections could be made to overcome the metabolic disturbances during pregnancy.

Conflict of interest statement

We declare that we have no conflict of interest.

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