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HORMONAL AND BIOCHEMICAL CHANGES DURING THE TRANSITION PERIOD IN HOLSTEIN FRISIAN COWS

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ABSTRACT

Dairy animals are very important for production in Egypt. The transition period is a critical period for animals which undergo different hormonal and biochemical changes. The present study was established to evaluate the effect of the transition period on some physiological and biochemical parameters in dairy cows. A number of 30 pregnant dairy Holstein cows were used for this study. Samples of animals were collected at the 2nd, 4th, and 6th week before calving, and at the 2nd, 4th, and 6th week after calving with a two-week interval. Also, samples from ration and water delivered to animals were collected for analysis of their contents. The result of the present study revealed a significant ($p<0.05$) increase in the level of progesterone, estrogen, prolactin, glucose, and MDA with a significant decrease in the level of cortisol, GSH, SOD, Ca, and P at the 2nd week before calving, compared to the 6th week before calving. There was a significant decrease ($p<0.05$) in the level of progesterone, estrogen, glucose, SOD, Ca, and P with a significant increase in insulin and prolactin levels at the 2nd week after calving compared to the 6th week before calving. Lastly, there was a significant increase in progesterone, estrogen, cortisol, glucose, and haptoglobin with a significant decrease ($p < 0.05$) in the levels of prolactin and GSH in the 2nd week after compared to the 6th week after calving.

Keywords: SOD, MDA, GSH, Haptoglobin& Ca.

INTRODUCTION

Dairy animals are considered important source of animal protein in Egypt. They are the main source of food supply in the form of meat and milk (FAO, 2009).

The animal transition period includes the change from a gestational non-lactating to a non-gestational lactating state (Morgante *et al.*, 2012). It is characterized by alterations in both immune functions and metabolism in

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animals (Piccione *et al.,* 2012). Some changes are related to the increase in the requirements of energy needed by the fetus and lactogenesis. It includes 3 weeks before and after calving (Contreras and Sordillo, 2011). In the dairy cow production cycle, the transition period is critical, as the metabolic and endocrine changes that accompany calving and the initiation of lactation. Lactation phases significantly affect the metabolic profile, so the differences recorded during various physiological phases are expected during the dairy cow production cycle (Smith and Risco, 2005).

Blood biochemical parameters differ during various physiological stages of animals (Ahmad *et al.,* 2003). The lactation stage is considered one of the most important stages in the life of dairy animals, which affects metabolism, resulting in the change in the haemato-biochemical profile (Iriadam, 2007). Due to the high economic importance of dairy animals, this study aimed to:

-Estimate the impacts of the transition period on estrogen, progesterone, prolactin, oxidant malonaldehyde (MDA) and antioxidants such as superoxide dismutase (SOD) and glutathione peroxidase (GPX), in addition to haptoglobin, glucose, cortisol, insulin, and some minerals (Ca, P, and Mg).

MATERIALS AND METHODS

This work was carried out at the farm of the Faculty of Agriculture, Sakha, Agriculture of Research Institute.

1. Animals

A total of 30 uniparous Holstein Frisian breed dairy cows aged about 3.5-4 years, weighing 550-620 kg, imported from Italy, America, and Germany, fed on a balanced ration, were apparently healthy, free from diseases, and internal and external parasites, and used for this experiment on the farm of Faculty of Agriculture, Sakha, Agriculture of Research Institute.

This work was done according to the ethics of the Faculty of Veterinary Medicine, South Valley University, with approval number (VM/SVU/23(2)-06).

2. Samples

2.1. Ration and water samples

The content of ration and water delivered for animals during the experiment was analyzed for their contents at the Chemistry and Nutritional Deficiency Department, Animal Health Research Institute, Giza, Egypt, and compared to the NRC.

2.2 Blood Sample.

The blood samples (7.5 ml) were collected from the jugular vein of each cow (n = 30) in clean glass vials after clipping and disinfecting the area of the vein, and then blood underwent spontaneous coagulation at room temperature. The blood sera were centrifuged at 3000 rpm and stored at -20℃ until testing, according to Coles (1986).

The separated sera are used for the detection of some minerals, including Mg, Ca, and P (mg/dl) (A.O.A.C., 2015), oxidants MDA (µmol/ml) (Ohkawa *et al.,* 1979), antioxidants included SOD (u/ml) (Nishikimi *et al.,* 1972) and GSH (u/ml) (Paglia and Valentine, 1967); these were estimated calorimetrically by means of test kits supplied by the Bio-diagnostic company, Egypt, using a spectrophotometer (Spectro UV-VIS Double beam PC scanning spectrophotometer UVD-2950), according to the manufacturer's instructions. Glucose (Young, 2001) by using a (Spectro UV-VIS Double beam PC scanning spectro-photometer UVD-2950). Insulin levels were determined using the ELISA method, according to A.O.A.C. (2015). Cortisol level was measured by using the competitive enzyme-linked immunosorbent assay (ELISA) kit (ADI-900-071, Enzo Life Sciences, Lausen, Switzerland) according to Palme and Mostl (1997). Haptoglobin level was determined by using bovine

haptoglobin (HP) ELISA kits with catalogue Nrs. (HAP-11, Life Diagnostics, Inc., West Chester, Pennsylvania), according to the manufacturer's instructions. Estrogen, progesterone, and prolactin were measured using ELISA kits, according to Titez (1994, 1995) and Vanderpump *et al.* (1998).

2.3. Statistical analysis

The data obtained were statistically analyzed according to Snedecor and Cochran (1980) for significance, analysis of variance by-one-way ANOVA using the statistical package for social science (SPSS) computer program.

RESULTS

1- The results of the rations and water analysis were as follows:

Table 1: Ration analysis.

Items	Before calving	After calving
Protein	16%	20%
Fat	3.5%	4%
Humidity	10%	10%
Ash	11%	11%
Fiber	13%	12%
Carbohydrates	46.5%	43%
Energy (calory/kg)	2873	2952
Calcium	2.1%	3%
Phosphorus	0.8%	0.8%
Magnesium	0.72%	1%

Table 2: Ration according to NRC.

Items	Before calving /ton	After calving /ton
Corn	650	550
Bran	250	150
Soya bean	100	100
Minerals	5	5
Vitamins	2.5	2.5
Carbohydrates	12.5	12.5
Nacl	5	5
Limestone	-	100
Rice hay	-	100
Alfa alfa		

Table 3: Water analysis

Items	Examined sample	Permissible limits (EOS) (1589/2007)
Appearance color	No color	Clear
Odor	Odorless	Odorless
PH value	7.8	6.5-8.5
Conductivity (ms/m)	110	2000(microms/m)
Calcium (mg/l)	50	Up to 150
Calcium carbonate (mg/l)	125	Up to 350
Magnesium mg/l	7	Up to 50
Magnesium carbonate	24	Up to 150
Total hardness as ca $Caco_3$	149	Up to 500
TSS (total suspended solids)	40	Up to 250
TDS (total dissolved solids)	320	Up to 750
TS (total solids)	360	Up to 1000
Chloride (mg/l)	20	Up to 250
Ammonia (mg/l)	-ve	Up to 0.5
Nitrate (mg/l)	-ve	Up to 0.2
Sulphate (mg/l)	38	Up to 250
Phosphorus (mg/l)	-ve	--
Phosphate (mg/l)	-ve	--
Total alkalinity	100	Up to 100
Bicarbonate alkalinity	100	
Carbonate Alkalinity	0	
Hydroxide alkalinity	0	

(EOS) Permissible limits by Egyptian Organization for Standardization & Quality

2- Effect of transition period on hormonal level

-Progesterone and estrogen levels

There was a significant increase in progesterone and estrogen levels in the 2nd week before calving and a significant decrease in their levels in the 2nd, 4th, and 6th weeks after calving, compared to their levels at the 6th week before calving, while there was a significant increase in their levels at the 2nd and 4th weeks after calving, compared to their levels at the 6th week after calving (P value ≤ 0.05), as shown in Table (4).

-Prolactin level

There was a significant increase in prolactin level in the 2nd week before and after calving, and the 4th and 6th weeks after calving compared to its level at the 6th week before calving, while a significant decrease in its level at the 2nd week after calving compared to the 6th week after calving at the (P value ≤ 0.05) (Table 4).

Table 4: Effect of the transition period on hormonal level

Calving time / Parameters	Before calving			After calving			P value
	6th week	4th week	2nd week	2nd week	4th week	6th week	
Progesterone (ng/ml)	71± 1.4	83± 1.5	91±1[a]	42± 1[ab]	22± 2.4[ab]	11.5±1[a]	0.001
Estrogen (ng/ml)	170±5.3	177± 10	181±7[a]	70±4.5[ab]	41±2[ab]	11±0.6[a]	0.001
Prolactin (ng/ml)	11±0.3	12.5±0.5	17±2[a]	21±0.8[ab]	24±0.5[a]	25±0.5[a]	0.001

Data presented as mean ± SD

-the letter a was used for significant differences of all groups after and before calving with the 6th week before calving group at the level of P≤0.05 at the same raw

-the letter b was used for significant difference in the 2nd & 4th week after calving groups, with the 6th week after calving group at the level of P≤0.05 at the same raw

3- Effect of the transition period on glucose, insulin and cortisol

-Glucose level

There was a significant decrease in glucose levels in the 2nd week before calving and the 2nd, 4th, and 6th weeks after calving, compared to the 6th week before calving. Also, there was a significant decrease in the 2nd week after calving, compared to the 6th week after calving (P≤0.05) (Table 5).

-Insulin level

There was a non-significant change in the level of insulin in all groups before calving compared with the 6th week before calving and all groups after calving compared to the 6th week after calving while, there was a significant increase in the level of insulin in the 2nd, 4th& 6th groups after calving compared with its level at 6th week before calving (P value ≤ 0.05) (Table 5).

-Cortisol level

There was a significant increase in cortisol level in the 2nd week before calving and a significant decrease in the 6th week after calving compared to the 6th week before calving, while there was a significant increase in its level in the 2nd and 4th week after calving compared with the 6th week after calving (P value ≤ 0.05) (Table 5).

Table 5: Effect of transition period on glucose, insulin and cortisol levels

Calving time / parameters	Before calving			After calving			P value
	6th week	4th week	2ndweek	2nd week	4thweek	6th week	
Glucose (mg/ml)	66±3	62±3	60±1.7[a]	59±2[ab]	55±2[a]	52±2[a]	0.001
Insulin (uIu/ml)	25±0.5	25±0.9	24±1	31±1.6[a]	32±2.3[a]	31±1.1[a]	0.001
Cortisol (nmol/ml)	14±1.1	14.4±1.1	16±1.5[a]	14±2[b]	13±2[b]	11.6±1[a]	0.001

Data presented as mean ± SD

4-Effect of the transition period on some oxidant and antioxidant levels

-MDA level

There was only a significant increase in MDA level in the 2nd week before calving compared to the 6th week before calving, while there was a non-significant change in the level of MDA in the 2nd and the 4th after calving compared to its level at the 6th week

before and after calving (P value ≤ 0.05) (Table 5).

-GSH level

There was a significant decrease in the GSH level at the 2nd and the 4th week before calving compared to its level at the 6th week before calving, while there was a significant decrease in its level at the 2nd week after compared to the 6th week after calving (P value ≤ 0.05) (Table 5).

-SOD level

There was a significant decrease in the level of SOD in the 2nd and the 4th week after and before calving compared to the 6th week before calving, while there was a non-significant change in groups compared to the 6th week after calving at the level of P value ≤ 0.05 as shown in (Table 5).

Table 6: Effect of transition period on some oxidant and antioxidants levels

Period / Parameters	Before calving			After calving			P value
	6th week	4th week	2nd week	2nd week	4th week	6th week	
MDA (µmol/l)	8.4±0.8	9±1.5	11±0.5[a]	10±0.2	9±0.9	8±0.2	0.001
GSH (mu/ml)	41±1.6	37±3[a]	32±2.2[a]	39±1[b]	42±1.5	45±1.3	0.001
SOD (u/ml)	7±0.3	6±0.4[a]	5±0.2[a]	5.9±0.4[a]	6±0.2[a]	6.4±0.3	0.001

5-Effect of the transition period on some minerals level

-Calcium& phosphorus levels

There was a significant decrease in the 2nd week before and the 2nd, 4th, and 6th weeks after calving compared to the 6th week before calving for both calcium and phosphorus minerals, while there was a non-significant change in groups after calving compared to the 6th week after calving at the level of P value ≤0.05 as shown in (Table 7).

-Magnesium level

There was a non-significant change in groups, compared to the 6th week before and after calving (P value ≤ 0.05) (Table 7).

Table 7: Effect of transition on some mineral levels

Period / Parameters	Before calving			After calving			P value
	6th week	4th week	2nd week	2nd week	4th week	6th week	
Ca (mg/ml)	9.3±0.2	9±0.1	8.5±0.3[a]	8.4±0.1[a]	8.5±0.2[a]	8.3±0.3[a]	0.001
P (mg/ml)	5.2±0.2	4.9±0.2	4.3±0.3[a]	4.2±0.1[a]	4.4±0.2[a]	4.4±0.2[a]	0.001
Mg (mg/ml)	3.1±0.1	2.9±0.2	2.8±0.2	2.7±0.2	3±0.3	3±0.2	0.058

6-Effect of the transition period on haptoglobin level

There was a significant decrease in haptoglobin level in the 4th and the 6th week after calving compared to its level at the 6th week before calving and a significant increase in its level at the 2nd and the 4th week after calving compared to the 6th week after calving (P value ≤0.05) (Table 8).

Table 8: Effect of transition period on haptoglobin level.

Calving time / Parameters	Before calving			After calving			P value
	6th week	4th week	2nd week	2nd week	4th week	6th week	
Haptoglobin (hpg/l)	43±1.3	43.4±1.1	45±1	42±1.2^{b}	40.4±1.1^{ab}	37±1.1^{a}	0.001

DISCUSSION

Dairy animals have merits for physiological adaptation to the high energy demands during the transition period, especially after calving for milk production. However, the individual animals vary tremendously in their adaptive success during this period (Sundrum, 2015). The transition period is one of the most critical periods in an animals' life, as cows undergo many physiological changes in order to prepare themselves for the onset of lactation till they reach the high peak of milk production (Elshahawy and Abdullaziz, 2017).

The results of the obtained study revealed that there was a significant increase in progesterone and estrogen levels in the 2nd week before calving. This result is in agreement with that reported by Ozgo and˙Skrzypczak (2000), who reported an increase in progesterone and estrogen levels in dairy animals, and this may be related to their function in maintaining pregnancy, the development of the mammary gland, and the onset of lactation (Kurpinska and Skrzypczak, 2019).

There was a significant decrease in the progesterone level in the 2nd, 4th, and 6th weeks after calving compared to its level at the 6th week before calving. This result is agreed with that reported by Kindahl *et al.* (2004) and Cernescu *et al.* (2010). This result may be due to the high level of the onset of lactation in the mammary gland (Kindahl *et al.*, 2002).

Our data revealed that there was a significant increase in progesterone level at the 2nd and 4th week after calving compared to its level at the 6th week after calving. This agreed with that reported by Kindahl *et al.* (2004), and this may occur due to ovulation.

The result of increased estrogen levels before calving came in accordance with that reported by Herdt (2000), who proved increased estrogen levels in dairy animals. This increase during the perinatal period is related to the preparation of the mammary gland for lactation and increased enzymatic activity of the mammary gland (Convey, 1973, and Kurpinska and Skrzypczak, 2019).

The increase of estrogen levels after calving then decrease agreed with that reported by Kindahl *et al.* (2004).

The result of the obtained study revealed that there was a significant increase in prolactin level in the 2nd week before and after calving and the 4th and 6th weeks after calving compared to its level at the 6th week before calving.

These results before and after calving agreed with those reported by Kurpinska and Skrzypczak (2019). This may be attributed to prolactin˙s important function in the secretion of milk during lactation; also, it is involved in the differentiation, development, and function of dairy cows' mammary tissue (Ollivier-Bousquet and Devinoy, 2005).

The result of the obtained study revealed that there was a significant decrease in prolactin levels in the 2nd week after calving compared to the 6th week after calving.
This result agreed with that reported by Kurpinska and Skrzypczak (2019), and this may be due to the production of milk and colostrum.

The result of our study revealed that there was a significant decrease in glucose levels in the 2nd week before calving compared to the 6th week before calving. These results agreed with those reported by Fiore *et al.* (2015) and Omur *et al.* (2016) and in contrast with those reported by El-Maghraby and Mahmoud (2016).

The end of pregnancy and the start of lactation represent a critical time as there is a massive increase in the need for glucose. This poses an enormous challenge for the liver, which has to synthesize all of this glucose from propionate and amino acids, as well as a challenge for other tissues and organs that have to adapt to the decrease of glucose availability (Tabrizi *et al.,* 2007). The development of the fetus and mobilization of maternal glucose to fetal blood circulation during advanced pregnancy and a high demand for lactose synthesis and/or insufficient gluconeogenesis during the period of early lactation (Jacob and Vadodaria, 2001) all ends with the reduction of glucose level.

The result of the obtained study revealed that there was a significant decrease in glucose levels in the 2nd week after calving compared to the 6th week after calving.

Cernescu *et al.* (2010) proved the same result, and this may be attributed to volatile fatty acids deficient in their synthesis at the rumen, especially propionic acid, which is essential for the gluconeogenesis process in case of insufficient glucidic intake (Cernescu *et al.,* 2010), or may be attributed to the high demand for energy needed by dairy animals for use in colostrum and milk production; also, this probably happened due to the needs of fat and lactose for milk production (Debski *et al.,* 2017).

The result of our study revealed that there was a significant increase in the level of insulin in the 2nd, 4th, and 6th groups after calving compared to its level at the 6th week before calving. The same results were reported by Moorby *et al.* (2000) and Ghanem *et al.* (2012), while these disagreed with that reported by Collet *et al.* (2019). Hypoglycemia is a common feature in dairy animals, as almost all the glucose is taken up by the udder for synthesis of lactose, and therefore, a dysregulation in insulin response develops to prioritize the glucose use by the mammary gland (De Koster and Opsomer, 2013), which takes place independently. As the energy requirements of the increasing milk yield are not met by diet in the first period after calving, the cow must use its own energy reserves.

The result of this study revealed that there was a significant increase in cortisol levels in the 2nd week before calving compared to the 6th week before calving. This result agreed with that reported by Herdt *et al.* (2000) and disagreed with that reported by Salah Eldein *et al.* (2023), and this result may be related to the stress of pregnancy, as the first biomarker of stress increase in animals is cortisol, so its level increases during pregnancy and lactation (Odore *et al.,* 2004).

There was a significant increase in cortisol levels in the 2nd and 4th week after calving compared to the 6th week after calving. This result is in accordance with that reported by Nikolic *et al.* (2003), and this is related to the stress of lactation, and with time this stress is relieved, and the animal accommodates with the lactation stage, as mentioned by Silva Filho *et al.* (2017).

There was a significant decrease in cortisol level at the 6th week after calving compared to the 6th week before calving this related to the absence of stress of pregnancy, and the animal accommodated with the lactation stage.

The result of the obtained study revealed that there was only a significant increase in MDA level in the 2nd week before calving compared to the 6th week before calving.

Elshahawy and Abdullaziz (2017) reported the same result, and this result disagreed with that reported by Castillo *et al.* (2005). The increase in MDA level before calving is due to the stress of calving, initiation of lactation, and colostrum production (Elshahawy and Abdullaziz, 2017).

There was a non-significant change in the level of MDA in the 2nd and 4th after calving groups compared to its level at the 6th week after calving. This may be attributed to the absence of stress of pregnancy, as MDA is considered the main indicator of stress accompanied pregnancy and lactation in dairy cows (Guo *et al.*, 2018).

The result of the obtained study revealed that there was a significant decrease in GSH and SOD levels at the 2nd and 4th week before calving compared to their levels at the 6th week before calving. This result agreed with that reported by Singh *et al.* (2017).

The decrease in antioxidant levels before calving was coincident with the decrease of minerals and vitamins supplementation, absorption in comparison with their need, along with the stress of heavy pregnancy and the onset of calving and lactation, and the decrease after calving may be attributed to the stress of lactation and colostrum production, while this decrease does not last for a long time (6th week) as the animal adapts itself for the production of milk (Elshahawy and Abdullaziz, 2017).

There was a significant decrease in SOD level at the 2nd and 4th week after calving compared to its level at the 6th week before calving, and this may be attributed to the negative energy balance during the postpartum period, which led to the promotion of fat mobilization and fatty acids oxidation (Ashmawy, 2015) that is considered a fundamental source of free radicals exceeding the normal antioxidant scavenging capacity (Li *et al.*, 2016).

There was a significant decrease in the 2nd week before and the 2nd, 4th, and 6th weeks after calving compared to the 6th week before calving. These results agreed with that reported by Singh *et al.* (2017) and Salah Eldein *et al.* (2023) and disagreed with that reported by Ghanem *et al.* (2012).

Zeinab (2007) and Hagawane *et al.* (2009) suggested that deficiency of minerals may be attributed to the insufficient mobilization of Ca from the skeleton, the improper food metabolite absorption from the gastrointestinal tract, excessive Ca loss in urine, and draining of a high percent of Ca and P.

There was a non-significant change in the level of Mg before and after calving, which may be attributed to the balanced ration delivered to animals during pregnancy and lactation. These results agreed with those reported by Cernescu *et al.* (2010) and Fiore *et al.* (2017) and disagreed with those reported by Singh *et al.* (2017).

The result of this study revealed that there was a significant decrease in haptoglobin level in the 4th and 6th weeks after calving compared with its level at the 6th week before calving.
Haptoglobin is an α2-globulin acute phase protein synthesized in the liver and formed in different species of domesticated animals (Baghshani *et al.* 2010). Its concentration increased with inflammation and stressful situations (Murata, 2007), so its decrease after calving may be related to the stress of pregnancy ending.

There was a significant increase in the haptoglobin level at the 2nd and 4th week after calving compared to the 6th week after calving, as it could be due to the stress of milking and colostrum production, and by the time (at the 6th week) the animal accommodates with the stress of the production of milk.

CONCLUSION

In this study we highlighted the hormonal and biochemical changes that occurred during the transition period in dairy cows; there was a significant increase in the level of progesterone, estrogen, prolactin, and glucose, MDA and a decrease in the level of cortisol, GSH, SOD, Ca, and P at the 2nd week before calving compared to the 6th week before calving; a decrease in the level of progesterone, estrogen, glucose, SOD, Ca, and P; and an increase in insulin and prolactin at the 2nd week after calving compared to the 6th week before calving; and an increase in progesterone, estrogen, cortisol, glucose, and haptoglobin. There was a significant decrease in prolactin and GSH at the 2nd week after compared to the 6th after calving. We recommend managing the dairy cows carefully during the transition period in order to avoid the occurrence of metabolic disorders and their subsequent reproductive failure.

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التغيرات الهرمونية والكيموحيوية أثناء الفترة الانتقالية في الابقار الهولشتاين الفريزيان

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تعتبر الحيوانات المنتجة للالبان من أهم مصادر الانتاج فى مصر. وتعتبر المرحلة الانتقالية من المراحل الحرجة في فترة حياة هذه الحيوانات والتي يحدث فيها العديد من التغيرات الهرمونية والكيموحيوية. تهدف هذة الدراسة الي تقييم تأثير الفترة الانتقالية على بعض العناصر الهرمونية والكيميائية في هذة الحيوانات. تم اختيار ٣٠ بقرة حالبة هولشتاين فريزيان من أجل هذة الدراسة عمرها يتراوح ما بين ثالثة سنوات الي اربعه. تم تجميع عينات سيرم في الاسبوع الثاني والرابع والسادس قبل وبعد الولادة بفارق زمني اسبوعين. تم تجميع عينات من الماء والعليقة المقدمة للحيوانات محل الدراسة لمعرفة مكوناتها. أوضحت مقارنة نتائج الاسبوع الثاني قبل الولادة بالأسبوع السادس قبل الولادة وجود زيادة في مستوي هرمونات الاستروجين، البروجسترون، هرمون اللبن الي جانب زيادة في مستوي الجلوكوز، والمالونالدهيد. وعلي النقيض وجود نقص في مستوي الكورتيزول، الجلوتاثيون السوبرأوكسيد ديسميوتيز وعنصري الكالسيوم والفسفور. عند مقارنة نتائج الاسبوع الثانى بعد الولادة بالاسبوع السادس قبل الولادة أوضحت النتائج وجود نقص في مستوي هرمون الاستروجين والبروجسترون ، الجلوكوز، السوبرأوكسيد ديسميوتيز، وعنصري الكالسيوم والفسفور وزيادة في مستوي هرموني الانسولين واللبن الي جانب وجود زيادة في مستوي هرمون الاستروجين، البروجسترون، الكورتيزول والجلوكوز والهبتاجلوبين. وفي النهاية بمقارنة الاسبوع الثاني بعد الولادة بالسادس بعد الولادة وجد زيادة في مستوى هرمون اللبن والجلوتاثيون.