

## Alteration of serum haptoglobin concentration in normal parturition and dystocia affected cows

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### Abstract

This study was designed to investigate the alteration in serum haptoglobin (Hp) concentration in dystocia affected cows in comparison to those having normal parturition. Thirty cows included in this study, seventeen with normal parturition and thirteen with difficult parturition (dystocia). Blood samples, from cows that having normal parturition, were collected every three days during the last two weeks of gestation, at calving, 3, 7, and 10 days postpartum. Blood samples from dystocia affected cows were collected at calving, 3, 7, and 10 days postpartum. Serum Hp concentration was determined using ELISA. The results gradual increase of Hp concentration prepartum from  $190 \pm 70$  mg/L at the 10<sup>th</sup> day before calving to  $250 \pm 30$  and  $260 \pm 100$  mg/L at 7<sup>th</sup> and 3<sup>rd</sup> day prepartum, respectively. At day of parturition, Hp concentration increased to  $300 \pm 140$  mg/L, to reach  $330 \pm 150$  mg/L at 3<sup>rd</sup> day postpartum. Hp concentration at 7<sup>th</sup> and 10<sup>th</sup> day postpartum declined to  $230 \pm 90$  and  $220 \pm 160$  mg/L, respectively. Serum Hp of dystocia affected cows was  $360 \pm 240$  mg/L at calving. At 3<sup>rd</sup> day postpartum, Hp increased to  $660 \pm 220$  mg/L. At 7<sup>th</sup> and 10<sup>th</sup> days postpartum, Hp concentration declined to  $510 \pm 300$  and  $400 \pm 110$  mg/L, respectively. No variation in Hp was observed between the cows giving twins and those giving single calve. There was no significant effect of dystocia causes on serum Hp at calving and at 3<sup>rd</sup> day postpartum. It be concluded that normal calving and dystocia elevated the serum Hp concentration, and insufficient dilatation of birth canal was the most effective cause of dystocia. Also comparison of serum Hp concentration at 3<sup>rd</sup> and 7<sup>th</sup> day postpartum can be used as an indicator for the development of complications.

**Keywords:** Cows, Dystocia, Haptoglobin, Parturition

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### التغيرات في تركيز الهابتوكلوبين في مصل دم الأبقار ذات الولادة الطبيعية والأبقار التي تعاني من عسر الولادة

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### الخلاصة

أجريت هذه الدراسة للتحري عن التغيرات في تركيز الهابتوكلوبين في مصل دم الأبقار التي تعاني من عسر الولادة بالمقارنة مع تلك ذات الولادة الطبيعية. استخدمت ثلاثون بقرة في هذه الدراسة، 17 بقرة لها ولادة سوية و 13 بقرة عانت من عسر الولادة. جمعت عينات الدم من الأبقار ذات الولادة الطبيعية كل ثلاثة أيام خلال الأسبوعين الأخيرين من الحمل، وكذلك يوم الولادة وفي الأيام 3 و 7 و 10 بعد الولادة. كما جمعت العينات من الأبقار التي عانت من عسر الولادة يوم الولادة وفي الأيام 3 و 7 و 10 بعد الولادة. تم قياس تركيز الهابتوكلوبين باستخدام اختبار الـ ELISA. بينت النتائج أن تركيز الهابتوكلوبين قد ازداد تدريجياً من  $190 \pm 70$  ملغم/لتر في اليوم 10 قبل الولادة إلى  $250 \pm 30$  و  $260 \pm 100$  ملغم/لتر في اليوم 7 و 3 قبل الولادة، وعلى التوالي. في يوم الولادة ارتفع تركيز الهابتوكلوبين إلى  $300 \pm 140$  و  $330 \pm 150$  ملغم/لتر واستمر ارتفاع مستواه ليصل إلى  $660 \pm 220$  ملغم/لتر في اليوم الثالث بعد الولادة. في اليوم السابع والعاشر بعد الولادة انخفض تركيز الهابتوكلوبين إلى  $510 \pm 300$  و  $400 \pm 110$  ملغم/لتر، على التوالي. أما تركيز الهابتوكلوبين في الأبقار التي عانت

من عسر الولادة فقد كان  $240 \pm 360$  ملغم/لتر يوم الولادة. في اليوم الثالث بعد الولادة ارتفع تركيز الهابتوكلوبين الى  $220 \pm 660$  ملغم/لتر. في اليوم السابع والعاشر بعد الولادة إنخفض تركيز الهابتوكلوبين الى  $300 \pm 510$  و  $110 \pm 400$  ملغم/لتر، على التوالي. لم يلاحظ وجود اختلاف في مستوى الهابتوكلوبين بين الابقار التي حملت توأما" والآخرى التي حملت عجل واحد. كما لم يكن هناك تأثير لسبب عسر الولادة على تركيز الهابتوكلوبين في يوم الولادة وكذلك في اليوم الثالث بعد الولادة. يستنتج من الدراسة ان الولادة الطبيعية وعسر الولادة ترفع تركيز الهابتوكلوبين، وأن عدم توسع قناة الولادة كان اكثر اسباب عسر الولادة تأثيرا على صحة الابقار. كذلك امكانية اعتماد المقارنة بين تركيز الهابتوكلوبين في اليوم الثالث والسابع بعد الولادة كدليل على حصول المضاعفات.

## Introduction

Acute phase proteins are group of glycoproteins, which secreted in response to infection, inflammation, trauma and injuries (1). The function of acute phase proteins is to promote production of immunoglobulin, enhancing tissue repair and limiting the damage caused by the infection or inflammation (2). Haptoglobin (Hp) is one of an important acute phase protein. It produced mainly in the liver but it is also produced by lung, adipose tissue, skin, spleen, udder, ovary, uterus and placenta (3-5). Additional to the biological function of Hp in responses to infection and inflammation (6), Hp has other functions such as antioxidant, antibacterial and anti-inflammatory (7-9).

The concentration of serum Hp has been determined in farm animals as an indicator for infection and cells damage. Also it was used as a prognostic indicator for many affections (10-17).

Parturition is a physiological event that includes hormonal and anatomical changes in genital system. Dystocia is defined as abnormal or difficult parturition, which can increase calf losses, cow mortality, delay return to estrus, and decrease conception rates (18).

This study was designed to investigate the alteration in serum Hp concentration in dystocia affected cows in comparison to those having a normal parturition.

## Materials and methods

### Animals

Thirty cows, aged between 4 and 7 years, were included in this study, seventeen with normal parturition and thirteen with difficult parturition (dystocia). Ten healthy heifers, aged between 7 to 10 months were included as a control group. The cows were bred individually by ten different farmers.

### Blood samples

Blood samples were collected once time from cows of control group. Samples from cows that having normal parturition were collected every three days during the last two weeks of gestation, at calving, 3, 7, and 10 days postpartum. Samples from dystocia affected cows were collected at calving, 3, 7, and 10 days postpartum.

Blood samples were kept at room temperature for 30 min to clot, then were kept at  $5^{\circ}\text{C}$  for 24 hours. Serum was collected by centrifugation at 3000 rpm for 15 min. Serum samples were stored at  $-20^{\circ}\text{C}$  until assay.

### Hp assay

Serum Hp concentration was determined using ELISA as method was described previously by Hiss *et al.* (19). Standard curve (Figure 1) was prepared to calculate the Hp concentration in samples.

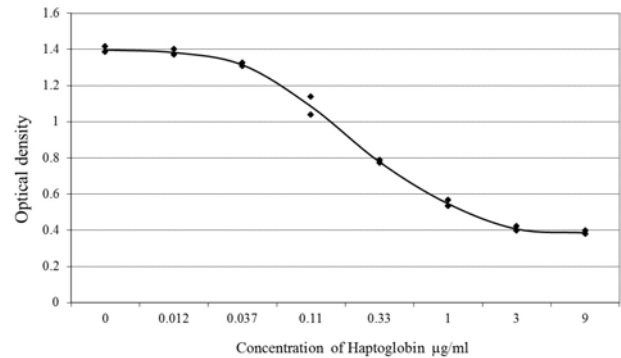


Figure 1: Standard curve of haptoglobin concentration.

### Statistical analysis

Data of study were presented as mean  $\pm$  SD. T-test and one way analysis of variance (followed by Duncan's multiple range test) were used to determine the significant differences between groups. SigmaStat (Jandel scientific software V3.1) was used for statistical analyses and  $P < 0.05$  was considered as statistically significant.

### Results

The mean value of serum Hp concentration in cows of control group was  $150 \pm 70$  mg/L (ranged from 50 to 230 mg/L).

Figure 2 shows the concentration of Hp in cows with normal parturition during the period between the 10<sup>th</sup> day prepartum and the 10<sup>th</sup> day postpartum. Hp concentration prepartum showed insignificant gradual increase from  $190 \pm 70$  mg/L at the 10<sup>th</sup> day prepartum to  $250 \pm 30$  and  $260 \pm 100$  mg/L at 7<sup>th</sup> and 3<sup>rd</sup> day prepartum, respectively. At

day of parturition, Hp concentration increased significantly ( $P<0.05$ ) to  $300\pm140$  mg/L, and it continually increased significantly ( $P<0.01$ ) to reach  $330\pm150$  mg/L at 3<sup>rd</sup> day postpartum. Hp concentration at 7<sup>th</sup> and 10<sup>th</sup> day postpartum declined to  $230\pm90$  and  $220\pm160$  mg/L, respectively.

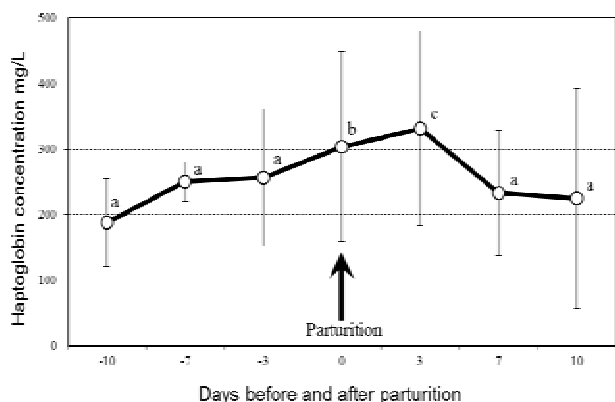


Figure 2: Haptoglobin concentration (Mean±SD) of normal calving cows (n = 17) during the period around calving. Means marked by different letters at each time were statistically different ( $P<0.05$ ).

Table 1 summarize the results of Hp concentration in dystocia affected cows in comparison to those having normal calving. Serum Hp concentration of dystocia affected cows was  $360\pm240$  mg/L at calving. No significant variation in Hp concentration at calving was observed between cows having normal and difficult calving.

At 3<sup>rd</sup> day postpartum, Hp concentration in cows having dystocia increased to  $660\pm220$  mg/L. This level was significantly ( $P<0.01$ ) higher than Hp concentration at calving also higher than the concentration at 3<sup>rd</sup> day postpartum in normal calving cows.

At 7<sup>th</sup> and 10<sup>th</sup> days postpartum, Hp concentration declined to  $510\pm300$  and  $400\pm110$  mg/L, respectively. These levels were significantly ( $P<0.05$ ) higher than Hp concentration at 7<sup>th</sup> and 10<sup>th</sup> day postpartum in normal calving cows.

At calving, Hp concentration in dystocia affected cows showed no significant variation between the cows that giving twins ( $320\pm80$  mg/L) and those giving single calve ( $250\pm90$  mg/L). At 3<sup>rd</sup> day postpartum, Hp concentration in both groups of cows increased significantly ( $P<0.05$ ) compared with that recorded at calving, but no variation was observed between these two groups (Table 2).

Hp concentration in cows having dystocia due to fault orientation, insufficient dilatation of birth canal and uterine inertia, were  $280\pm60$ ,  $320\pm120$  and  $190\pm110$  mg/L, respectively. These levels increased significantly ( $P<0.05$ ) at 3<sup>rd</sup> day postpartum in dystocia cases caused due to fault orientation and insufficient dilatation of birth canal but not due to uterine inertia. There was no significant effect of dystocia causes on serum Hp at calving and at 3<sup>rd</sup> day postpartum (Table 3).

Table 1: Haptoglobin concentration (mean ± SD) in normal calving cows (n=17) and dystocia affected cows (n=13)

Calving and postpartum days	Haptoglobin concentration mg/L	
	Normal calving	Dystocia
0	$300 \pm 140$	$360 \pm 240$
3	$330 \pm 150$	$660 \pm 220$ **
7	$230 \pm 90$	$510 \pm 300$ *
10	$220 \pm 160$	$400 \pm 110$ *

\*\* Significant variation ( $P<0.01$ ), \* Significant variation ( $P<0.05$ ) between normal calving and dystocia affected cow.

Table 2: Haptoglobin concentration (mean ± SD) in dystocia affected cows which having single and twins birth

Type of birth	No. of cows	Hp concentration mg/L	
		At calving	3 days postpartum
Single	10	$250 \pm 90$	$520 \pm 180$ *
Twins	3	$320 \pm 80$	$670 \pm 230$ *

\* Significant variation ( $P<0.05$ ) between the concentration at calving and 3<sup>rd</sup> day postpartum.

Table 3: The effect of dystocia causes on haptoglobin concentration (mean ± SD) in cows

Causes of dystocia	No. of cows	Hp concentration mg/L	
		At calving	3 days postpartum
Fault orientation	7	$280 \pm 60$	$640 \pm 170$ *
Insufficient dilatation of birth canal	3	$320 \pm 120$	$810 \pm 230$ *
uterine inertia	3	$190 \pm 110$	$390 \pm 170$

\* Significant variation ( $P<0.05$ ) between the concentration at calving and 3<sup>rd</sup> day postpartum.

## Discussion

The results indicated that no detectable variation in Hp concentration during the pregnancy. This result agree with previous studies which found no effect of pregnancy on Hp concentration (16,20).

There was an increase in Hp concentration during the last week of gestation. This result was observed previously in cows and mares (11,21). These changes in serum Hp may be occur due to the physiological changes before parturition, especially elevation of cortisone at this period of gestation (18,22). It was reported that increasing serum cortisone concentration is followed by increasing Hp concentration (21).

The results showed that serum Hp increased significantly at calving day. Same observation was recorded in cows (23), mares (11), ewes (12), and does (14). Elevation of serum Hp at calving may be occur due to the changes in estrogen level at this period, it was recorded that Hp level increased with increases of estrogen concentration (11).

Hp was increased significantly at 3<sup>rd</sup> day postpartum, after that it declined at 7<sup>th</sup> day and returned to normal value at 10<sup>th</sup> day postpartum. Peak Hp concentration was observed at 3<sup>rd</sup> day postpartum because it was produced in response to the uterine tissue damage during parturition (24). Decreasing Hp level at 7<sup>th</sup> day postpartum and later can be attributed to the low production of Hp and destruction of serum Hp during this period because the half-life of Hp is 3.5 days (25).

Hp in dystocia affected cows was significantly higher especially at 3<sup>rd</sup> day postpartum, which could reflect the degree of tissue damage in birth canal. Dystocia cases are more susceptible to injuries and trauma in birth canal than normal birth (18).

## Conclusions

It be concluded that normal calving and dystocia elevated the serum Hp, and insufficient dilatation of birth canal was the most effective cause of dystocia on health of cows. Also comparison of serum Hp at 3<sup>rd</sup> and 7<sup>th</sup> day postpartum can be used as an indicator for the development of complications.

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## References

1. Burtis CA, Ashwood ER. Tietz textbook of clinical chemistry. 3<sup>rd</sup> ed. London: Saunders Company, 1999: p. 494-497.
2. Kent JE. Acute phase proteins: Their use in veterinary diagnosis. *Br Vet J* 1992;148,279-282.
3. Kalmovarin N, Friedrichs WE, O'Brien HV, Linehan LA, Bowman BH, Yang F. Extrahepatic expression of plasma protein genes during inflammation. *Inflammation* 1991;15,369-379.
4. Friedrichs WE, Navarajo-Ashbaugh AL, Bowman BH, Yang F. Expression and inflammatory regulation of haptoglobin gene in adipocytes. *Bioch Biophys Res Com* 1995;209,250-256.
5. Yang F, Friedrichs WE, Navarajo-Ashbaugh AL, deGraffenried LA, Bowman BH, Coalson JJ. Cell type-specific and inflammatory-induced expression of haptoglobin gene in lung. *Lab Inv* 1995;73,433-440.
6. Dobryszyska W. Biological function of haptoglobin-new pieces to an old puzzle. *Eur J Clin Chem Clin Bioch* 35, 647-654
7. Eaton JW, Brandt P, Mahoney JR, Lee JT. Haptoglobin: a natural bacteriostat. *Science* 1982;215,691-693.
8. Jue DM, Shim BS, Kang YS. Inhibition of prostaglandin synthase activity of sheep seminal vesicular gland by human serum haptoglobin. *Mol Cell Bioch* 1983;51,141-147.
9. Gutteridge JM. The antioxidant activity of haptoglobin towards haemoglobin-stimulated lipid peroxidation. *Bioch et Biophys Acta* 1987;917,219-223.
10. Skinner JG, Brown RAL, Roberts L. Bovine haptoglobin response in clinically defined field conditions. *Vet Rec* 1991;128,147-149.
11. Taira I, Fujinaga T, Okumura M. Equine haptoglobin: isolation, characterization, and the effects of ageing, delivery, inflammation on the serum concentration. *J Vet Med Sci* 1992;54,435-442.
12. Aziz DM, Taha MB. Effect of dystocia on serum haptoglobin in Awassi ewes. *Theiogenology* 1997;48,559-562.
13. Lipperheide C, Goth C, Petersen B, Sommer H. Nephelometric assay of haptoglobin in blood plasma from cattle, pigs and horses. *Tierarztliche Umschau* 1997;52,420-426.
14. Al-Sultan MAH, Aziz DM. Serum haptoglobin in caprine dystocia. *Iraqi Journal of Veterinary Sciences* 1998;11,237-239.
15. McGrotty YL, Knottenbelt CM, Ramsey IK, Reid SWJ, Eckersall PD. Haptoglobin concentrations in a canine hospital population. *Vet Rec* 2003;152,562-564.
16. Nazifi S, Rezakhani A, Koohimoghadam M, Ansari- Lari M, Esmailnezhad Z. Evaluation of serum haptoglobin in clinically healthy cattle and cattle with inflammatory disease in shiraz, a tropical area in southern Iran. *Bulg J Vet Med* 2008;2,95-101.
17. Nazifi S, Saeb M, Ghasemian O, Esmailnezhad Z. Evaluation of serum haptoglobin in clinically healthy Iranian camels (*Camelus dromedarius*). *Comp Clin Path* 2006;15,195-197.
18. Noakes DE, Parkinson TJ, England GCW. *Arthur's Veterinary Reproduction and Obstetrics* (8<sup>th</sup> ed.). China: Elsevier Ltd, 2001: p. 152-335.
19. Hiss S, Mielenz M, Bruckmaier RM, Sauerwein H. Haptoglobin concentrations in blood and milk after endotoxin challenge and quantification of mammary Hp mRNA expression. *J Dairy Sci* 2004;87,3778-3784.
20. Huzzey JM, Duffield TF, LeBlanc SJ, Veira DM, Weary DM, von Keyserlingk MAG. Haptoglobin as an early indicator of metritis. *J Dairy Sci* 2009;92,621-625.
21. Uchida E, Katoh N, Takahashi K. Appearance of haptoglobin in serum from cows at parturition. *J Vet Med Sci* 1993;55,893-894.
22. Jackson PGG. *Handbook of veterinary obstetrics* (2<sup>nd</sup> ed.). London: W. B. Saunders, 2004: p. 37-80, 209- 231.
23. Drillich M, Voigt D, Forderung D, Heuwieser W. Treatment of acute puerperal metritis with flunixin meglumine in addition to antibiotic treatment. *J Dairy Sci* 2007;90,3758-3763.
24. Berkova N, Lemay A, Dresser DW, Fontaine JY, Kerizit J, Goupil S. Haptoglobin is present in human endometrium and shows elevated levels in the deciduas during pregnancy. *Mol Human Reprod* 2001;7,747-754.
25. Sadrzadeh SMH, Bozorgmehr J. Haptoglobin phenotypes in health and disorders. *Am J Clin Pathol* 2004;121,97-104.