



ORIGINAL ARTICLE

Detection of haptoglobin in seminal plasma of Awassi rams and the relation with its level in serum and some semen parameters



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KEYWORDS

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Abstract The study was conducted to detect haptoglobin in seminal plasma (SP-Hp) of Awassi rams and the effect of the breeding season on its concentration, along with determining the correlation with its concentration in serum (S-Hp) and main semen variables. Pre-warmed artificial vagina was used to collect semen samples biweekly from five Awassi rams. Semen samples were evaluated for volume, concentration and sperm motility. Blood samples were collected 10–30 min after semen collection. The concentration of serum and seminal plasma Hp was determined using ELISA. The mean \pm SESP-Hp concentrations ranged from 0.25 ± 0.05 to 0.81 ± 0.44 μ g/ml, whereas those of S-Hp ranged from 0.99 ± 0.29 to 2.99 ± 0.18 mg/ml. There was a significant ($P < 0.05$) positive correlation ($r = 0.329$) between SP-Hp and S-Hp concentrations. Both SP-Hp and S-Hp concentrations were significantly ($P < 0.05$) higher in winter as compared with the other seasons. The concentrations of SP-Hp and S-Hp during the breeding season were significantly lower ($P < 0.01$) than those of the out season period. SP-Hp concentration negatively correlated with semen volume and sperm concentration ($r = -0.164$ and -0.121), whereas sperm concentration positively correlated with individual sperm motility ($r = 0.100$). No significant correlation was detected between SP-Hp and semen parameters. It can be concluded that, Hp is present in ram seminal plasma and its concentration was about 2000 folds lower than that of the serum, and Hp concentration was lower during the breeding season, but its concentration in seminal plasma has no significant correlation with semen parameters.

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Introduction

Haptoglobin (Hp) is an acute phase protein, which is mainly produced in the liver as well as – other tissues such as lung [1], adipose tissue [2], skin [3], spleen [4], udder [5], ovary, uterus and placenta [6]. The most important biological function of – Hp includes host defense responses to infection and inflammation [7]; it acts as an antioxidant [8], antibacterial

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[9], anti-inflammatory [10], inhibitor of prostaglandin synthesis [11] and it has an effect on vitamin C metabolism [12].

Hp has been detected in the serum of human and many farm animal species including equine, bovine, ovine, caprine and swine. Furthermore, it has also been detected in the milk [13–16], saliva [17], urine [18], meat juice [19], and uterine secretion [20–22].

Previous studies have reported Hp detection in the reproductive system of women and in females of some laboratory and farm animals; women uterus [22] and follicular fluid [23], rat ovaries [24], mice uterus and ovaries [25], rabbit uterus [6], and buffalo follicular fluid [26].

There is no information on the presence of Hp in male reproductive system of farm animals. One study, however, recorded the production of Hp by Sertoli cells of rats [24]. Therefore, the present study was conducted to detect Hp in seminal plasma of Awassi rams and the effect of breeding season on its concentration, and to determine the correlation of its concentration in serum and some semen parameters.

Material and methods

Animals

Five adult rams, 3.5–4 years of age were used in this study. The rams were maintained at the animal house of the College of Veterinary Medicine, University of Mosul under uniform feeding and housing conditions.

Collection of seminal plasma

Semen samples were collected biweekly using pre-warmed artificial vagina (40 °C) during the period from July 2010 to February 2011 (18 ejaculates from each ram). During this period, the rams were passing through the breeding season (July – September) and non-breeding season (October – February) [27]. Immediately after semen collection, each sample was individually evaluated for volume using a graduated test tube, individual sperm motility by light microscopy and sperm concentration using a spectrophotometer [28].

Seminal plasma was collected by dilution of the semen samples with physiological normal saline at a ratio of 1:10, then plasma was separated using a centrifuge at 3000g for 30 min. Seminal plasma samples were kept at –20 °C until assayed.

Blood collection

Ten to thirty minutes after semen collection blood samples were collected using 18 gauge needles into sterile serum separation tubes. The samples were allowed to clot at room temperature for 30 min, and then kept at 5 °C for 24 h. Thereafter, the blood tubes were centrifuged at 3000g for 15 min and the serum was collected and stored at –20 °C until assayed.

Hp assay

The concentration of serum and seminal plasma Hp was determined using ELISA according to the method that was previously described by Hiss et al. [5]. The Hp concentration in samples was calculated according to the prepared standard curve (Fig. 1).

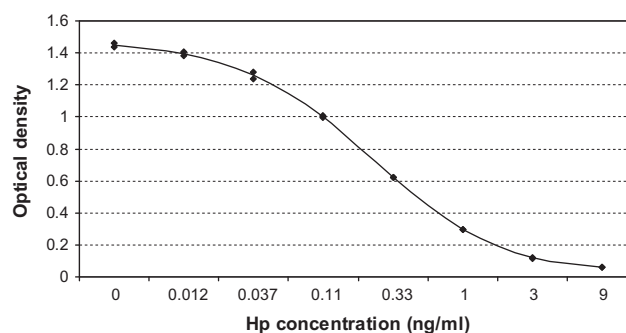


Fig. 1 Standard curve of Hp concentration.

Statistical analysis

Data of all experiments were expressed as mean \pm SE. Data were statistically analyzed by one way analysis of variance, followed by Duncan's multiple range test. Pearson correlation coefficients was used to analyze the relationship between the parameters. All statistical analyses were performed by Sigma Stat (Jandel Scientific Software V3.1). $P < 0.05$ was considered as statistically significant.

Results

Semen parameters

The mean \pm SE of semen volume of the rams along the period of study was 1.23 ± 0.05 ml, and the sperm concentration was $3.88 \pm 0.06 \times 10^9$ sperm/ml, whereas that of the individual motility was $87.66 \pm 1.04\%$.

Ram individual variation

The mean seminal plasma Hp concentration of the five rams during study period ranged (from) 0.25 ± 0.05 (to) 0.81 ± 0.44 μ g/ml. No significant difference was detected among the SP-Hp of the rams. The serum Hp concentration of ram during the period of study ranged (from) 0.99 ± 0.29 (to) 2.99 ± 0.18 mg/ml. A significant ($P < 0.05$) difference was observed in S-Hp concentration among the rams (Table 1). There was a significant ($P < 0.05$) positive

Table 1 The individual variation between rams in SP-Hp and S-Hp concentration (Mean \pm SE, 18 ejaculates and blood samples from each ram).

No. of ram	SP-Hp (μ g/ml)	S-Hp (mg/ml)
1	0.81 ± 0.44^a	1.05 ± 0.26^a
2	0.25 ± 0.05^a	0.99 ± 0.29^a
3	0.60 ± 0.23^a	2.99 ± 0.18^b
4	0.48 ± 0.11^a	1.43 ± 0.43^a
5	0.62 ± 0.11^a	1.17 ± 0.23^a

A significant ($P < 0.001$) difference was observed in S-Hp concentration between rams.

^{a,b} The different letters at the columns refer to significant difference ($P < 0.05$).

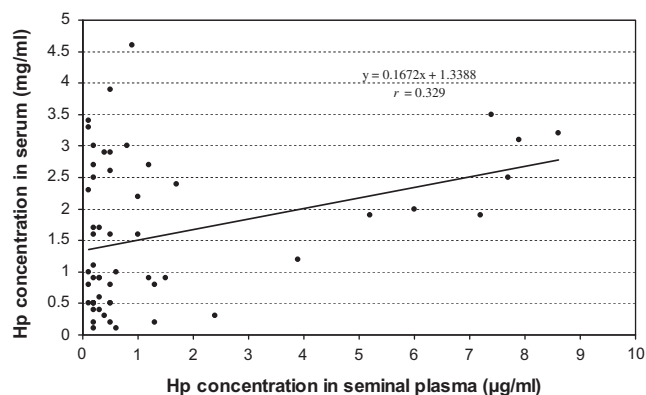


Fig. 2 Correlation between concentration of SP-Hp and S-Hp ($n = 5$).

correlation ($r = 0.329$) between SP-Hp and S-Hp concentration (Fig. 2).

Season of year

SP-Hp concentration was significantly higher ($P < 0.05$) in winter ($0.53 \pm 0.10 \mu\text{g/ml}$) compared to those of the summer ($0.29 \pm 0.02 \mu\text{g/ml}$) and autumn ($0.36 \pm 0.06 \mu\text{g/ml}$). No significant variation occurred in SP-Hp between summer and autumn. The S-Hp concentration varied significantly ($P < 0.01$) among the seasons of the year. The concentration in winter was higher ($2.16 \pm 0.22 \text{mg/ml}$) than those of the summer ($0.40 \pm 0.12 \text{mg/ml}$) and autumn ($1.45 \pm 0.22 \text{mg/ml}$) (Table 2).

Breeding season

SP-Hp and S-Hp concentrations ($0.27 \pm 0.04 \mu\text{g/ml}$ and $0.71 \pm 0.30 \text{mg/ml}$) during the breeding season were significantly lower ($P < 0.01$) than those of the out season period ($0.63 \pm 0.10 \mu\text{g/ml}$ and $1.65 \pm 0.26 \text{mg/ml}$) (Table 3).

Seminal plasma Hp and semen parameters

SP-Hp concentration negatively correlated with semen volume and sperm concentration of ram ($r = -0.164$ and -0.121), whereas its concentration positively correlated with individual sperm motility ($r = 0.100$). However, the correlation was not significant between SP-Hp and semen parameters (Table 4).

Table 2 Seasons of year and SP-Hp and S-Hp concentration (Mean \pm SE, $n = 5$).

Season of year	SP-Hp ($\mu\text{g/ml}$)	S-Hp (mg/ml)
Summer	0.29 ± 0.02^a	0.40 ± 0.12^a
Autumn	0.36 ± 0.06^a	1.45 ± 0.22^b
Winter	0.53 ± 0.10^b	2.16 ± 0.22^c

A significant difference in SP-Hp ($P = 0.024$) and S-Hp ($P = 0.002$) concentration was recorded among the seasons of year, ^{a,b,c} The different letters at the columns refer to significant difference ($P < 0.05$).

Table 3 Breeding seasons and SP-Hp and S-Hp concentration (Mean \pm SE, $n = 5$).

Season	SP-Hp ($\mu\text{g/ml}$)	S-Hp (mg/ml)
Breeding season	$0.27 \pm 0.04^*$	$0.71 \pm 0.30^*$
Non-breeding season	0.63 ± 0.10	1.65 ± 0.26
<i>P</i> value	0.01	0.048

* There is a significant difference among the breeding seasons.

Table 4 Correlation coefficient between SP-Hp concentration and semen parameters ($n = 5$).

Semen parameters	Correlation coefficient (r) with SP-Hp concentration	<i>P</i> value
Semen volume	-0.164	0.192
Semen concentration	-0.121	0.336
Individual motility	0.100	0.426

Discussion

Hp was determined previously in some body fluids such as saliva, milk and urine [15–18], and the result of this study indicated the presence of Hp in ram seminal plasma.

The concentration of serum Hp was more than 2000 folds higher than that of the seminal plasma. The recorded concentration of Hp in this study is lower than that of the porcine saliva [17] and higher than that of the bovine milk [29].

The significant correlation between serum and seminal plasma Hp concentrations in accordance with the results of others in which a significant correlation was reported between serum Hp and saliva and meat juice Hp of swine [17] and milk Hp of cows [13,14].

Results of the present study indicated that Hp in serum and seminal plasma was higher during winter when compared with autumn and summer. This variation could occur as a result of cold stress during the winter months. Up to our knowledge, there is no available reference studying the effect of the seasonal changes on Hp concentration in domestic animals. However, one report indicated that Hp concentration was higher in winter than in spring in European brown bears [30].

This study indicated that, the level of Hp in serum and seminal plasma was lower during the breeding season. The reason for this finding is not clear, although it could occur as a result of elevated testosterone level during the breeding season of rams [31]. Borglin and Nyman [32] have recorded a negative correlation between women serum Hp and serum concentration of estrogen.

The volume and sperm concentration of semen reflect the health status of male reproductive system; therefore, theoretically semen samples having a good volume and concentration must have low Hp concentration in seminal plasma. Our results confirm this theory, there was a negative relationship between Hp concentration in seminal plasma, semen volume and sperm concentration. The same relationship was also observed between milk Hp concentration and health status of udder [5,13–16].

Conclusion

Hp is present in ram seminal plasma and its concentration was about 2000 folds lower than that of the serum, and Hp concentration was lower during the breeding season, but its concentration in seminal plasma has no significant correlation with semen parameters.

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References

- [1] Yang F, Ghio A, Herbert D, Weaker F, Walter C, Coalson J. Pulmonary expression of the human haptoglobin gene. *Am J Respir Cell Mol Biol* 2000;23:277–82.
- [2] do Nascimento C, Hunter L, Trayhurn P. Regulation of haptoglobin gene expression in 3t3-11 adipocytes by cytokines, catecholamines, PPAR gamma. *Biochem Biophys Res Commun* 2004;313:702–8.
- [3] Wang H, Gao X, Wang Y, Li P, He C, Xie Y, Chen H. Expression of haptoglobin in human keratinocytes and langerhans cells. *Br J Dermatol* 2005;153:894–900.
- [4] D'Armiento J, Dalal S, Chada K. Tissue, temporal and inducible expression pattern of haptoglobin in mice. *Gene* 1997;195:19–27.
- [5] 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;87:3778–84.
- [6] Olson G, Winfrey V, Matrisian P, Melner M, Hoffman L. Specific expression of haptoglobin mRNA in implantation-stage rabbit uterine epithelium. *J Endocrinol* 1997;152:69–80.
- [7] Dobryszczyka W. Biological functions of haptoglobin—new pieces to an old puzzle. *Eur J Clin Chem Clin Biochem* 1997;35:647–54.
- [8] Gutteridge JM. The antioxidant activity of haptoglobin towards haemoglobin-stimulated lipid peroxidation. *Biochim Biophys Acta* 1987;917:219–23.
- [9] Eaton JW, Brandt P, Mahoney JR, Lee JT. Haptoglobin: a natural bacteriostat. *Science* 1982;215:691–3.
- [10] Jue DM, Shim BS, Kang YS. Inhibition of prostaglandin synthase activity of sheep seminal vesicular gland by human serum haptoglobin. *Mol Cell Biochem* 1983;51:141–7.
- [11] Sadrzadeh SMH, Bozorgmehr J. Haptoglobin phenotypes in health and disorders. *Am J Clin Pathol* 2004;121:97–104.
- [12] Langlois MR, Delanghe JR, De Buyzere ML. Effect of haptoglobin on the metabolism of vitamin C. *Am J Clin Nutr* 1997;66:606–10.
- [13] Eckersall PD, Young FJ, McComb C, Hogarth CJ, Safi S. Acute phase proteins in serum and milk from dairy cows with clinical mastitis. *Vet Rec* 2001;148:35–41.
- [14] Grönlund U, Hulten C, Eckersall PD, Hogarth C, Persson Waller K. Haptoglobin and serum amyloid A in milk and serum during acute and chronic experimentally induced *Staphylococcus aureus* mastitis. *J Dairy Res* 2003;70:379–86.
- [15] Grönlund U, Hallén Sandgren C, Persson Waller K. Haptoglobin and serum amyloid A in milk from dairy cows with chronic sub-clinical mastitis. *Vet Res* 2005;36:191–8.
- [16] Hiss S, Mueller U, Neu-Zahren A, Sauerwein H. Haptoglobin and lactate dehydrogenase measurements in milk for the identification of subclinically infected quarters. *Vet Med* 2007;52:245–52.
- [17] Hiss S, Knura-Deszczka S, Regula G, Hennies M, Gymnich S, Petersen B, Sauerwein H. Development of an enzyme immuno assay for the determination of porcine haptoglobin in various body fluids: testing the significance of meat juice measurements for quality monitoring programs. *Vet Immunol Immunopathol* 2003;96:73–82.
- [18] Roy R. Detection of haptoglobin from concentrated urine samples by enzyme immunoassay. *J Forensic Sci* 1991;36:115–21.
- [19] Gómez-Laguna J, Gutiérrez A, Pallarés FJ, Salguero FJ, Cerón JJ, Carrasco L. Haptoglobin and C-reactive protein as biomarkers in the serum, saliva and meat juice of pigs experimentally infected with porcine reproductive and respiratory syndrome virus. *Vet J* 2010;185:83–7.
- [20] Beier HM, Beier-Hellwig K. Molecular and cellular aspects of endometrial receptivity. *Hum Reprod Update* 1998;4:448–58.
- [21] Sharpe-Timms KL, Ricke EA, Piva M, Horowitz GM. Differential expression and localization of de-novo synthesized endometriotic haptoglobin in endometrium and endometriotic lesions. *Hum Reprod* 2000;15:2180–5.
- [22] Berkova N, Lemay A, Dresser DW, Fontaine JY, Kerizit J, Goupil S. Haptoglobin is present in human endometrium and shows elevated levels in the decidua during pregnancy. *Mol Human Reprod* 2001;7:747–54.
- [23] Porta A, Cassano E, Balestrieri M, Bianco M, Picone R, De Stefano C, Abrescia P. Haptoglobin transport into human ovarian follicles and its binding to apolipoprotein A-1. *Zygote* 1999;7:67–77.
- [24] O'Bryan M, Grima J, Mruk D, Cheng C. Haptoglobin is a Sertoli cell product in the rat seminiferous epithelium: its purification and regulation. *J Androl* 1997;18:637–45.
- [25] Friedrichs WE, Navarijo-Ashbaugh AL, Bowman BH, Yang F. Expression and inflammatory regulation of haptoglobin gene in adipocytes. *Biochem Biophys Res Commun* 1995;209:250–6.
- [26] Bergamo P, Balestrieri M, Carratore V, Abrescia P. Purification of a 240 kDa protein from serum and follicular fluid of water buffalo and its identification as haptoglobin. *J Exp Zool* 1995;271:452–61.
- [27] Zarkawi M. Monitoring the reproductive performance in Awassi ewes using progesterone radioimmunoassay. *Small Rum Res* 1997;26:291–4.
- [28] Bearden HJ, Fuquay JW, Willard ST. *Applied animal reproduction*. 6th ed. New Jersey: Pearson Prentice Hall; 2004, p: 173–93.
- [29] Khoshvaghti A, Nazifi S, Vafafar A, Gheisari HR. Evaluation of serum and milk haptoglobin in some inflammatory diseases of cattle. *Int J Vet Res* 2009;3:31–6.
- [30] Mominoki K, Morimatsu M, Karjalainen M, Hohtola E, Hissa R, Saito M. Elevated plasma concentrations of haptoglobin in European brown bears during hibernation. *Comp Biochem Physiol Part A: Mol Integrative Physiol* 2005;142:472–7.
- [31] Sanford LM, Palmer WM, Howland BE. Changes in the profiles of serum LH, FSH and testosterone and in mating performance and ejaculate volume in the ram during the ovine breeding season. *J Animal Sci* 1977;45:1382–91.
- [32] Borglin NE, Nyman M. Effect of estrogens on the haptoglobin level in the blood. *Scandinav J Clin Lab Invest* 1961;13:107–15.