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# Characterization of gender-specific bovine serum

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Animal cell cultures generally require a nutrient-rich medium supplemented with animal serum. Adult bovine serum contains a variety of nutrients including inorganic minerals, vitamins, salts, proteins and lipids as well as growth factors that promote animal cell growth. To evaluate the potential use of gender-specific bovine serum (GSBS) for cell culture, the biochemical properties of male serum (MS), female serum (FS) and castrated-male serum (CMS) were investigated. Overall, the chemical profile of GSBS was similar to that of bovine references except for glucose, creatine kinase, lactate dehydrogenase and potassium. FS showed elevated total protein and sodium concentrations compared to MS and CMS. Proteins present in MS, FS and CMS but absent in fetal bovine serum (FBS) were selected by two-dimensional gel electrophoresis and identified by peptide mass fingerprinting. Some of the identified proteins are known to be involved in immune responses and the others have unknown physiological roles. Moreover, it was found that some proteins such as alpha-2-macroglobulin appeared to be gender-specific with higher contents in FS. Insulin and testosterone was significantly higher in MS, and 17β-estradiol and estrone were higher in FS, as compared to the other sera. Taken together, the results indicate that each GSBS has a different ratio of components. Differences in serum constituents may affect cell cultures in a different manner and could be beneficial, depending on the specific aim of cell cultures.

**Keywords:** adult bovine serum; serum proteins; cell culture

## Introduction

Use of animal serum in culture media to reproduce in vivo conditions started with the development of cell culture technique in 1900s. Serum provides an optimum cell culture environment by providing albumin, steroids, cytokines and growth factors. In addition, serum helps to maintain pH, osmotic pressure and electrolytes that play critical roles in cell growth and morphology (Pilili et al. 2010). As examples, albumin helps in transport and acts as an antioxidant (Francis 2010), and vitronectin/fibronectin aids in cell spreading and heparin and collagen binding activity (Hayman et al. 1985; Aday et al. 2011). However, substitution of serum in cell culture media has not been successful due to the presence of ambiguous factors.

Endogenous hormones in animal sera, which vary according to gender, have a greater effect in vitro (Milo et al. 1976). Serum that is rich in testosterone enhances the myoblast formation of satellite and C3H10T1/2 cells (Sinha-Hikim et al. 2003). Estrogen and estrone enhance growth of breast cancer cells (Lee et al. 2009) and higher insulin content enhances the growth of vascular endothelial cells and leukocytes (Hirata et al. 2005). Steroid-free serum prepared using charcoal-dextran has been used to study the cellular effect of steroids. However, the charcoal-dextran process used to eliminate certain steroid components in the serum also removes some desirable components such as fatty acids, vitamins, electrolytes and metabolites essential for cell growth, and imparts additional variables to the experiment (Cao et al. 2009). Thus, gender-specific sera with high or low contents of testosterone and estrogen can provide an in situ environment for certain specific cell cultures.

In Korea, up to 650,000 cattle are slaughtered every year and around 25 million liters of blood waste is produced (Roça 2002). Only a small amount of bovine blood from slaughter-houses is used currently for the production of plasma proteins (Belhocine et al. 1998), probiotics (Chang and Shin 1998) and food additives. Steroids in blood are potent environmental contaminants and continuous exposure to them can lead to testicular, breast and prostate cancer, endometriosis, polycystic ovarian syndrome and decreased semen quality (Foster 2008). Therefore, recycling bovine blood in the preparation of serum for cell culture could help decrease the environmental burden. In addition, we have previously reported the advantage of using gender-specific bovine serum (GSBS) as

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compared with fetal bovine serum (FBS), at least in certain type of cell cultures (Lee et al. 2009). In the present work, we characterized the sera from male, female and castrated-male cattle.

## Materials and methods

### *Reagents and equipment*

All chemicals used in this study were of analytical grade and were purchased from Sigma-Aldrich Korea (Seoul, Korea) unless otherwise mentioned. Pharmalyte (pH 3.5–10) was from GE Healthcare Korea (Seoul, Korea) and IPG Dry Strips (pH 4–10 NL, 24 cm long) were from Genomine (Pohang, Korea). Modified porcine trypsin (sequencing grade) was from Promega Korea (Seoul, Korea). Analytical reagents and equipments used for serum analyses were BS-400 from Mindray (Shenzhen, China), Hemavet from CDC Technologies (Oxford, CT, USA) and EasyLyte from Medica (Bedford, MA, USA).

### *Serum isolation*

Blood samples from 47 bovine individuals (3–4-year-old females, $n=15$; 2-year-old males, $n=18$; 2-year-old castrated males, $n = 14$) were collected in sterile bottles from a local slaughter-house at Yeongcheon, Gyeongsanbuk-do, Republic of Korea. The blood samples were quickly stored at 4°C and serum was isolated according to Lee et al. (2009). Collected blood samples were quickly stored at 4°C for 4–5 h. The separated serum was collected in a sterile bottle and stored at −20°C for 24 h after centrifugation at 5000 rpm at 4°C for 20 min. The frozen serum was thawed and centrifuged at 7000 rpm for 20 min, and the supernatant was collected prior to inactivation at 56°C for 30 min. Finally, the serum was filtered through a 0.22 μm pore size filter for bacterial eradication and stored at −20°C until further use.

### *Chemical analysis*

All equipment used for serum chemical analyses was in routine use and was calibrated daily. The choices of analytical methods were based on the general veterinary clinical pathology practice (Table 1; Stockham and Scott 2008). All biochemical data were analyzed using SPSS version 12.0 (SPSS, Chicago, IL, USA). Statistical differences were compared by one-way ANOVA test followed by Bonferroni tests for multiple comparisons. Differences with a $P$ value $<0.05$ were considered as significant.

### *Hormone analysis*

Hormone level was analyzed according to Lee et al. (2010). Enzyme-linked immunosorbent assay (ELISA) kits for testosterone, estrone and 17β-estradiol (DRG Instruments GmbH, Margurg, Germany) were used for hormonal analysis. Standard controls of known concentration, or test serum and enzyme conjugate, were added in a specific-antibody-coated microtiter well and incubated for 1–2 h at room temperature. The mixture was removed and unbound samples were washed thrice with washing solution. After adding substrate solution for 15–30 min, the enzymatic reaction was terminated by adding stop solution. The absorbance at 450 nm was measured within 10 min using an ELISA plate reader (Microplate autoreader; Bio-Rad, Hercules, CA, USA). A bovine insulin ELISA kit from ALPCO Diagnostics (Salem, NH, USA) was used for quantitative analysis of insulin following manufacture's protocol.

### *Preparation of serum protein samples for two dimensional-polyacrylamide gel electrophoresis (2D-PAGE)*

Pooled serum samples of castrated ($n=14$), male ($n=18$) and female ($n=15$) cattle were prepared by mixing equal volumes of individual serum in each group. The pooled serum samples were dissolved in 7 M urea, 2 M thiourea containing 4% (w/v) 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS), 1% (w/v) dithiothreitol (DTT) and 2% (v/v) pharmalyte and 1 mM benzamidine. Proteins were extracted for 1 h at room temperature with vortexing. After centrifugation at 15,000 × $g$ for 1 h at 15°C, insoluble material was discarded and the soluble fraction was used for 2D-PAGE. Protein loading was normalized as previously described (Bradford 1976).

### *2D-PAGE and quantitative analysis of image*

IPG dry strips were equilibrated for 12–16 h with 7 M urea, 2 M thiourea containing 2% CHAPS, 1% DTT, 1% pharmalyte, and loaded with 1 mg of the protein samples prepared as above. Isoelectric focusing (IEF) was performed at 20°C using a Multiphor II electrophoresis unit and EPS 3500 XL power supply (Amersham Biosciences, Piscataway, NJ, USA) following the manufacturer's instructions. For IEF, the voltage was linearly increased from 150 to 3500 V during 3 h for sample entry followed by a constant 3500 V, with focusing complete after 96 kVh. Prior to electrophoresis in the second dimension, strips were incubated for 10 min in equilibration buffer (50 mM Tris-Cl, pH 6.8, containing 6 M urea, 2% sodium dodecyl sulfate (SDS) and 30% glycerol), first with 1% DTT and second with

Table 1. Reagents and equipment used for blood analysis.

| Test | Analytical method | Reagent | Equipment |
|---|---|---|---|
| Total protein | Biuret | Cormary[1] | BS-400[2] |
| Albumin | Bromocresol green colorimetric | ELItech[3] | BS-400 |
| Aspartate aminotransferase | Kinetic | ELItech | BS-400 |
| Alanine transaminase | Kinetic | ELItech | BS-400 |
| Alkaline phosphatase | Kinetic | ELItech | BS-400 |
| Total cholesterol | Enzymatic | ELItech | BS-400 |
| Total bilirubin | Modified Malloy-Evelyn | ELItech | BS-400 |
| Glucose | Enzymatic | ELItech | BS-400 |
| Blood urea nitrogen | Enzymatic | ELItech | BS-400 |
| Creatinine | Jaffe's | Cormary | BS-400 |
| Creatine kinase | Kinetic | ELItech | BS-400 |
| Hemoglobin | Electrical impedence | Reagent for Hemavet 850FS | Hemavet 850FS[4] |
| Sodium | Ion selective electrode | Easy electrolytes[5] | EasyLyte[6] |
| Potassium | Ion selective electrode | Easy electrolytes | EasyLyte |
| Chloride | Ion selective electrode | Easy electrolytes | EasyLyte |
| Calcium | *o*-Cresolphthalein complexone assay | SIEMENS[7] | BS-400 |
| Phosphorus | U.V. end point | ELItech | BS-400 |

[1]Poland; [2]MINDRAY, CHINA; [3]France; [4]CDC Tech., USA; [5]Medica, USA; [6]USA.

2.5% iodoacetamide. Equilibrated strips were inserted onto SDS-PAGE gels ($20 \times 24$ cm, 10–16%). SDS-PAGE was performed using Hoefer DALT 2D system (Amersham Biosciences) following the manufacturer's instructions. All 2D gels were run at 20°C for 1700 Vh, and Coomassie G250 stained as previously described (Anderson et al. 1991). Quantitative analysis of digitized images was done using the PDQuest version 7.0 software (BioRad), according to the protocols provided by the manufacturer. The quantity of each spot was normalized by total valid spot intensity.

### *In-gel protein digestion*

Selected protein spots were digested in-gel using modified porcine trypsin in a manner similar to that previously described (Shevchenko et al. 1996). Gel pieces were washed with 50% acetonitrile to remove SDS, salt and stain, dried to remove solvent and then rehydrated with trypsin (8–10 ng/μl) and incubated 8–10 h at 37°C. The proteolytic reaction was terminated by addition of 0.5% trifluoroacetic acid (TFA). Peptides were recovered by combining the aqueous phase from several extractions of gel pieces with 50% aqueous acetonitrile. After concentration the peptide mixture was desalted using $C_{18}$ZipTips (Millipore, Billerica, MA, USA), and peptides were eluted in 1–5 μl of acetonitrile.

### *Peptide mass fingerprinting (PMF)*

An aliquot of peptides prepared as above was mixed with an equal volume of α-cyano-4-hydroxycinnamic acid in 50% acetonitrile/0.1% TFA, and subjected to matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) analysis using an the Ettan MALDI-TOF Pro apparatus (Amersham Biosciences, Piscataway, NJ, USA) as previously described (Fernandez et al. 1998). Spectra were collected from 350 shots per spectrum over an *m/z* range of 600–3000 and calibrated by two-point internal calibration using trypsin auto-digestion peaks (*m/z* 842.5099, 2211.1046). The peak list was generated using the Ettan MALDI-TOF Pro Evaluation Module version 2.0.16. The threshold used for peak-picking was 5000 for minimum resolution of monoisotopic mass and 2.5 for S/N. The search program MASCOT (http://www.matrixscience.com/) was used for protein identification by PMF. The following parameters were used for the database search: trypsin as the cleaving enzyme, a maximum of one missed cleavage, iodoacetamide (Cys) as a complete modification, oxidation (Met) as a partial modification, monoisotopic masses and a mass tolerance of $\pm$ 0.1 Da. The PMF acceptance criterion was probability scoring.

## Results

### *Biochemical profile*

Table 2 presents chemical profiles of male serum (MS), female serum (FS) and castrated-male serum (CMS). Most of the serum constituent levels were within the bovine reference range. However, glucose, creatine kinase, lactate dehydrogenase (LDH) and potassium showed higher average values in all three groups compared with the reference values. Although all three groups were within the reference ranges, total protein

Table 2. Summary of blood chemical profiles in male, female and castrated male cattle.

| | Reference range* (unit) | Male (range) | Female (range) | Castrated (range) |
|---|---|---|---|---|
| Total protein# | 5.7–8.1 (g/dl) | 7.4[a, b] (6.2–8.7) | 7.6[a] (6.8–9.6) | 7.0[b] (6.5–7.6) |
| Albumin | 3.0–4.3 (g/dl) | 3.7 (3.3–4.0) | 3.6 (3.4–4.0) | 3.6 (3.4–3.8) |
| Aspartate aminotransferase | 78–132 (u/l) | 92.5 (73.0–127.0) | 79.3 (48.0–103.0) | 74.8 (52.0–97.0) |
| Alanine transaminase | 11–40 (u/l) | 23.4 (13.0–28.0) | 20.9 (13.0–27.0) | 20.7 (12.0–28.0) |
| Alkaline phosphatase | 0–500 (u/l) | 109.7 (49.0–212.0) | 60.4 (32.0–101.0) | 89.3 (60.0–184.0) |
| Cholesterol | 65–220 (mg/dl) | 177.2 (119.0–272.0) | 162.6 (89.0–237.0) | 150.7 (84.0–240.0) |
| Bilirubin | 0.01–0.5 (mg/dl) | 0.2 (0.1–0.3) | 0.2 (0.1–0.5) | 0.2 (0.0–0.3) |
| Glucose† | 45–75 (mg/dl) | 164.0 (101.0–248.0) | 153.7 (54–322) | 179.3 (118–320) |
| Blood urea nitroge | 6.0–27.0 (mg/dl) | 14.2 (8.6–22.1) | 14.4 (11.0–23.3) | 14.0 (10.4–16.7) |
| Creatinine | 1.0–2.0 (mg/dl) | 1.4 (1.1–1.8) | 1.4 (0.1–1.9) | 1.3 (1.0–1.5) |
| Creatine kinase† | 105.0–409.0 (u/l) | 572.9 (340.0–854.0) | 507.9 (193.0–878.0) | 471.4 (192.0–1056.0) |
| Lactate dehydrogenase† | 692.0–1445.0 (u/l) | 4370.2 (3179.0–5506.0) | 4438.5 (3006.0–5847.0) | 4279.3 (2770.0–5637.0) |
| Hemoglobin | 8.0–15.0 (g/dl) | 13.8 (10.1–18.6) | 13.0 (11.1–15.5) | 13.5 (10.3–18.2) |
| Sodium# | 132–152 (mmol/l) | 144.6[a, b] (140.9–149.5) | 146.4[a] (141.2–152.4) | 144.3[b] (140.2–148.1) |
| Potassium† | 3.9–5.8 (mmol/l) | 8.0 (5.8–10.0) | 7.9 (4.7–10.0) | 8.3 (6.4–10.0) |
| Chloride | 95–110 (mmol/l) | 100.1 (96.8–102.5) | 101.3 (96.7–105.4) | 100.3 (97.2–103.5) |
| Calcium | 9.7–12.4 (mg/dl) | 9.4 (8.7–10.2) | 9.3 (8.3–10.7) | 9.5 (8.7–10.6) |
| Phosphorus | 5.6–6.5 (mg/dl) | 6.0 (4.4–8.1) | 5.9 (3.0–7.5) | 6.4 (4.3–9.8) |

*Data from Radostits et al. (2000).
†Concentrations in GSBS are significantly higher than the bovine reference range.
#Different superscript designates significant difference ($P < 0.05$).

and sodium concentrations in FS were slightly higher than in CMS (Table 2).

### *Identification of adult bovine-specific serum proteins*

The proteins in pooled MS, FS and CMS were separated by 2D-PAGE (Figure 1). Comparing the 2D gel images for the samples with the image obtained for a commercial FBS, 70 protein spots were found to be specific for MS, FS and CMS. By quantitative comparison of each protein spot, 22 protein spots that exhibited over a 2-fold variation were selected and classified in five gender-specific groups (Figure 2). PMF for the 22 proteins identified eight proteins (Table 3). Most of the identified proteins (CM-S1, CM-M-S3, CM-M-S5, CM-M-S6 and F-S1) were likely to be involved in immune responses. The protein CM-M-S2 was detected at a higher level in CMS and MS (2.9- and 3.8-fold, respectively, compared to the content in FS); the protein was identified as alpha-1B-glycoprotein. Protein CM-M-S7 was identified as a hemopexin-like protein, and elevated 3.2-fold and 2-fold in CMS and MS, respectively, compared to FS. Protein CM-F-S1 was identified as alpha-2-macroglobulin, which binds to and modulates growth factors, cytokines and hormones (Feige et al. 1996). The protein was expressed 2.7-fold and 2.0-fold more in CMS and FS, respectively, than in MS.

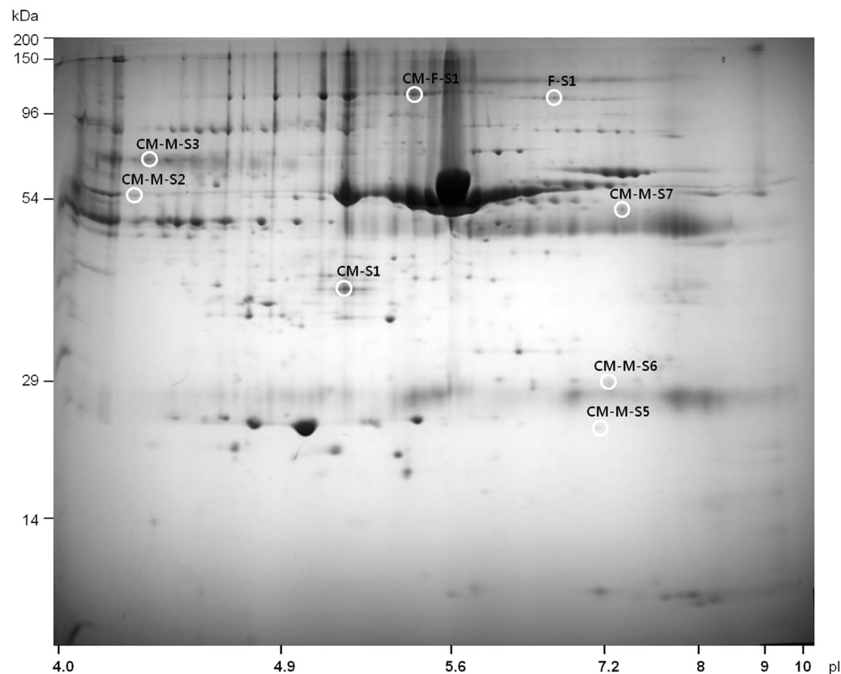


Figure 1. 2D-PAGE analysis of female serum. Serum protein samples were prepared and separated according to their isoelectric point (pI) and molecular mass (MW). Protein spots identified by PMF are indicated with circles and numbers, and summarized in Table 3.

***Hormonal analysis***

Testosterone was significantly higher in MS than in FS and CMS ($P < 0.01$), whereas estrogen levels (both 17β-estradiol and estrone) were highest in FS and lowest in CMS. The insulin level was significantly higher in MS compared to FS and CMS (Table 4).

## Discussion

In order to test the potential use of GSBS for cell culture, biochemical constituents, proteins and hormone in FS, MS, and CMS were investigated in this study. In general, the blood chemical profile of GSBS was compatible with other bovine strains. In GSBS, glucose, creatine kinase, LDH and potassium levels were higher than the reference ranges. It is most likely that elevated LDH and potassium concentrations were due to hemolysis, which is known to cause leakage of LDH and potassium from erythrocytes (Mather and Mackie 1960; Leung and Henderson 1981). Although we took great care while collecting blood samples during the slaughter process, hemolysis was unavoidable since the samples were collected from bleeding carcasses. The overall values for glucose and creatine kinase were higher than the reference range in all three sera. Traumatic injuries or surgical handling of viscera evoke neuroendocrine responses that resulted in hyperglycemia (Reis et al. 1998). Moreover, marked elevation of serum creatine kinase concentration is associated with stressful situations (Cockram and Corley, 1991). Thus, elevated glucose and creatine kinase levels in GSBS may reflect the extreme anxiety in cattle undergoing slaughter.

FS showed higher total protein and sodium concentrations than MS and CMS. Unfortunately, we were unable to find any previous observations that explain sex-related differences in total protein and sodium concentration in serum. Potential reasons could be pregnancy, since the majority of slaughtered cattle used in the study were 3–4 years of age, and may have already experienced several pregnancies. Thus, higher serum total protein might be related to the necessity for milk production (McAdam and O'Dell 1982). It has been shown that solutes in culture media significantly affect cell growth, with the major effect being reproduced by simply altering sodium concentration (Rubin and Chu 1984). Moreover, serum protein is a ready source of unknown growth factors that are likely to exert diverse effects on cells in culture. Therefore, elevated total protein and sodium concentrations should be taken into consideration when using female GSBS.

Although the currently identified proteins were selected to be gender-specific, they are more likely to be adult bovine-specific (and so not detected in FBS). In fact, proteomic analysis of blood sera is challenging due to the abundant proteins, such as albumin (up to 60% of total serum proteins) and immunoglobulin in

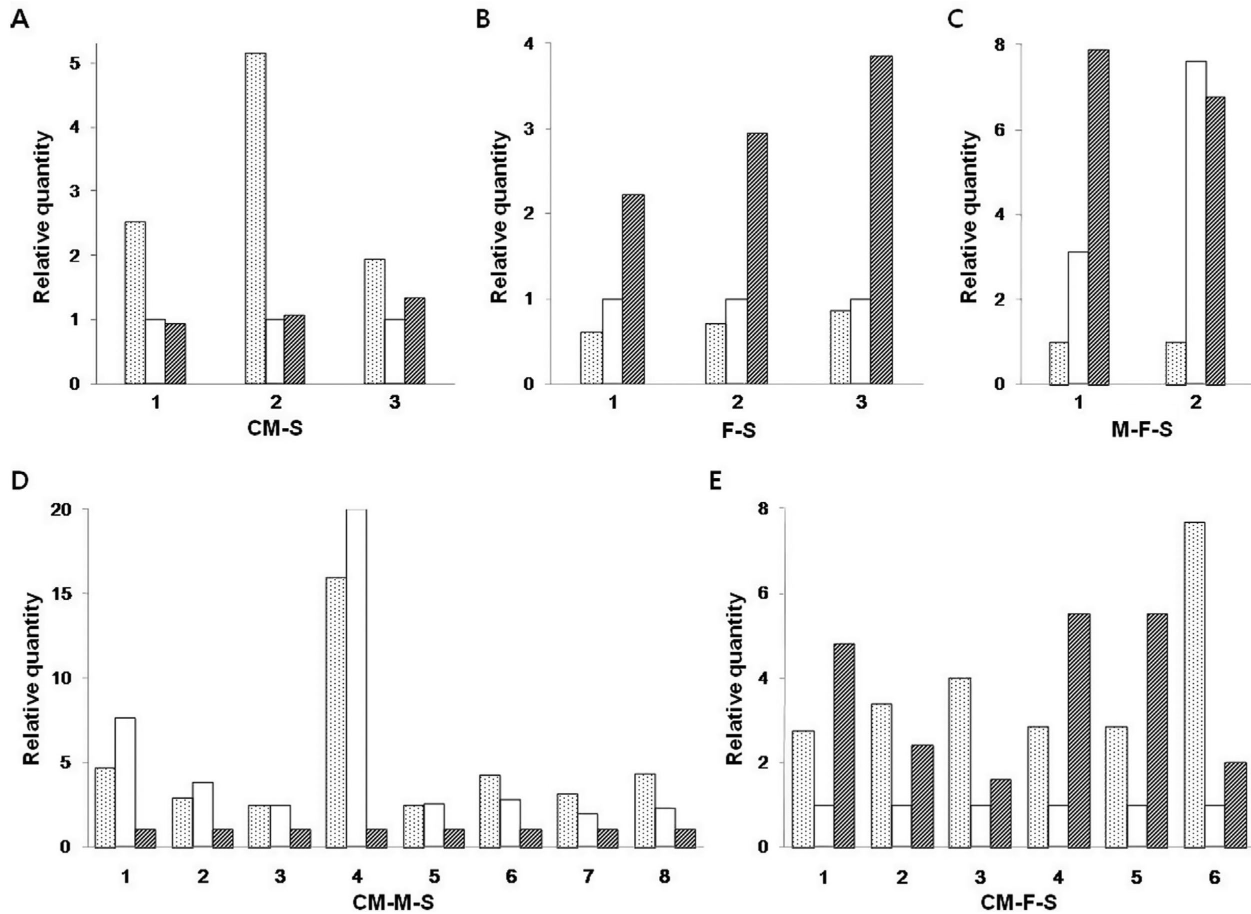


Figure 2. Quantitative analysis of putative gender-specific serum proteins. Seventy serum protein spots initially screened as specific for GSBS were quantitatively analyzed. Twenty-two proteins that exhibited variable deviation over 2-fold in quantity between each group of CMS (▩), MS (□) and FS (▨) were selected for further identification and grouped as castrated male-specific (CM-S, A), female-specific (F-S, B), male and female-specific (M-F-S, C), castrated male and male-specific (CM-M-S, D) and castrated male and female-specific (CM-F-S, E). The relative quantity was obtained by comparing spot intensity, and the number on the *x*-axis indicates the spot number in each group.

serum. Hence, the enrichment of minor proteins after removing abundant serum proteins would enhance the identification of more gender-specific proteins. Another problem might be the low cut-off value (2-fold in quantity comparing each protein in MS, FS and CMS) for the screening of gender-specific serum proteins. From the initial screening, some proteins were detected to deviate more significantly in quantity between each serum group and were more likely to be gender-specific. But the PMF identification was not

Table 3. Identification of proteins by PMF analysis.

| Spot # | Protein ID | Proteins | Sequence coverage (%) | Score | MW (Da) |
|---|---|---|---|---|---|
| CM-S1 | NP_001035559 | Complement C3 | 12 | 151 | 188652 |
| CM-M-S2 | NP_001039708 | Alpha-1B-glycoprotein precursor | 21 | 108 | 54091 |
| CM-M-S3 | AAB62251 | IgM H-chain constant region | 32 | 122 | 48512 |
| CM-M-S5 | NP_001137569 | C-reactive protein precursor | 22 | 89 | 25504 |
| CM-M-S6 | CAJ31249 | Prepro complement component C3 | 7 | 90 | 188715 |
| CM-M-S7 | NP_001029784 | Hemopexin precursor | 16 | 104 | 52974 |
| F-S1 | DAA21469 | Complement factor H precursor | 13 | 93 | 99830 |
| CM-F-S1 | NP_001103265 | Alpha-2-macroglobulin | 28 | 325 | 168953 |

PMF was performed as described in the Materials and methods for 22 protein spots selected in Figure 2. Using the MASCOT search program (http://www.matrixscience.com/), eight proteins were identified and summarized.

Table 4. Analysis of hormones in different genders of adult bovine sera.

| | T (ng/ml) | | E2 (pg/ml) | | E1 (pg/ml) | | Insulin (ng/ml) | |
|---|---|---|---|---|---|---|---|---|
| Female | 1.16 | (0.31–2.07) | 100 | (24–279)[a] | 420 | (105–1468) | 0.45 | (0.05–2.46) |
| Male | 10.76 | (4.78–18.62)[a] | 69 | (13–255) | 320 | (64–671) | 0.74 | (0.02–2.48)[a] |
| Castrated | 1.49 | (0.31–3.21) | 27 | (14–72) | 215 | (72–688) | 0.30 | (0.05–0.62) |

Hormone analysis was done as described in the Materials and methods by ELISA for testosterone (T), estrone ($E_1$), 17β-estradiol (E2) and insulin.
[a] < 0.01.

successful, probably due to the low amount of proteins in sera.

Nevertheless, our proteomic analysis identified adult bovine-specific and putative gender-specific proteins. More than half of the identified proteins (Table 3, components of complement, immunoglobulin) were shown to be directly involved in immune responses of adult bovines against potent pathogens encountered during their lifetimes. The protein alpha-1B-glycoprotein was grouped in castrated male and male-specific proteins (CM-M-S in Figure 2 and Table 3). Although its physiological role has not been clearly understood, the protein contains conserved immunoglobulin domains (Ishioka et al. 1986) and may be a marker for mycobacterial infections of cattle (Seth et al. 2009). Another castrated male and male-specific protein, CM-M-S7, was identified as a hemopexin-like protein that has 73% amino acid homology with hyaluronidase from porcine liver (Zhu et al. 1994), which catalyzes the hydrolysis of hyaluronic acid in extracellular matrix (Stern 2008). The most interesting protein identified in this report was alpha-2-macroglobulin, which was specific for proteinase inhibitors and binds growth factors and hormones as a carrier (Feige et al. 1996). The protein has autocrine or paracrine roles in increasing 17β-estradiol production by granulosa cells (Ireland et al. 2004). The amount of 17β-estradiol in FS was determined to be 1.5-fold that in MS (Table 4), which was coincident with the relative amount of alpha-2-macroglobulin. However, the amount of the same hormone in CMS did not agree with the alpha-2-macroglobulin analysis.

Hormone analysis revealed variation in hormonal level which is gender-specific. The level of testosterone observed in MS was significantly higher than in FS and CMS ($P < 0.01$). Gender variation in the level of testosterone may be due to the difference in nutritional status (Brito et al. 2007) and age; testosterone level increases each month up until puberty (Barth et al. 2008). Testosterone in MS may be one of the causes of high muscle proliferation shown by myogenic satellite cells compared to FBS (Lee et al. 2009). In contrast, the estrogen level was significantly higher in FS than in MS and CMS. Although the difference within the level of estrogen and estrone was seen in FS, it may be due to ovarian cyclic variation (Dobson et al. 1974). Again, the increased proliferation in breast cancer cells may be due to high estrogen level in female serum compared to FBS (Lee et al. 2009). In contrast, all testosterone, 17β-estradiol and estrone were lowest in castrated male cattle compared to both the male and female cattle.

It has been well-documented that muscle development during and after puberty in animals is closely associated with increased circulating sex steroids in blood secreted from the gonads. Increased amounts of sex steroids, directly or indirectly via the stimulation of other factors affecting muscle cell growth and differentiation, will eventually lead to muscle development in animals (Sinha-Hikim et al. 2003). In addition, responsiveness of certain cell types is greatly affected by gender-specific sex hormones. For example, estrogens are required for breast and uterine cancer cell growth, while androgens are required for prostate cancer cell growth. In this respect, our previous result that demonstrated enhanced cell proliferation and differentiation of myogenic satellite cells in MS as compared with FS, CMS as well as FBS highlights the importance of optimal serum for cell cultures. The data from this study suggest the possible application of GSBS as a substitute for FBS.

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