Comparison of serum protein profile in Indonesian Local Ettawah goats with single and twin offspring using SDS-PAGE

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Abstract. Indonesian Local Ettawah Goats (ILEG) is local Indonesian livestock with more than one offspring potential. There is no description of the serum protein profile of female goat with single and twin offspring. This study aimed to compare the protein band type, the percentage of protein band appearance, protein band thickness between the female goat serum of single and twin offspring. This research method was a case study at the breeding village of Ampel Gading Malang East Java, Indonesia. The sample came from ILEG female with single and twin offspring, which had a record of three offsprings with six replications per group. Serum samples were isolated from whole blood taken through the goat jugular vein. Separation of blood serum with Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) and performed by Panther Data Base for analysis of protein type data. The results showed that single and twin ILEG had ten types of protein bands 13-140 kDa with an average percentage of protein band appearances of 8.32% higher in twin offsprings compared to single offspring. The thickening level of a protein band at 44-94 kDa in female goat with twin offsprings was increased expression compared to single offspring. The ILEG protein profile of twin offspring was higher in quality and quantity than single offspring. The research recommends molecular protein weight at 44-94 kDa as a candidate to an early detect female goat with twin offspring.

Keywords: protein band, prolific, local goat

1. Introduction
Indonesian Ettawah Local Goat or ILEG [1,2] or also known as Ettawah Crossbred or PE is Indonesian germplasm which has distinctive morphological characteristics (long drooping ears, hair colour variations of white black or white brown, small horns, convex face). Besides that, ILEG also had a genetic similarity index among ILEG individuals > 70% and commonly repeated-G [3,4]. Other potentials ILEG has high adaptability to the management of small borders and resistance to disease, multiple purposes (meat and milk production) and fertility or animal breeding (having 1-3 kids per offspring) [3-5].
There are many genes influenced prolificacy, namely BMPR1B [5], BMP15 [6], GDF9 [6], POU1F1 [7], PRLR [8], KISS1 [9], GPR54 [10], Inhibin HA [7], BMP4 [11], IGF1 [12], GH [7], GHR [7], ESR1 [13], ESR2 [13], FSHB [7], FSHR [7] and GnRHR [7]. Follicle Stimulating Hormone Receptor (FSHR) and Gonadotropin-Releasing Hormone Receptor (GnRHR) were rhodopsin receptors that function as or like G protein-coupled receptors (GPCR). GPCR was an integral membrane protein that varies in size formed from 400 to 1000 amino acid residues [14].

Goats with twin offspring had higher concentrations of FSH and GnRH than goats with single offspring [15] followed by FSHR and GnRHR concentrations were also higher in goats of twin offsprings than single offspring, but no information about serum protein level of both. Another research of Saanen goats had proved that the protein profile of blood serum increased in the pregnant goats compared to non pregnant goats [16].

Therefore, it is necessary to research the profile of blood serum proteins to obtain a comparison of biomolecular expression of twins and single offspring does. The research aims were analyzed of blood serum protein to provide an overview of the type and percentage of protein band, band appearance, protein band thickness between single and twin offspring ILEG.

2. Material and Method
This research method was a case study with a descriptive analysis. This study used ILEG raised in Ampel Gading District, Malang Regency with a natural mating system. ILEG female goats (doe) selection was based on following criteria: had given birth at least 3 times with single or twin offsprings, feeding and management housing was standard.

Sample preparation. Blood sampling for each replication in each group was through the jugular vein as much as 5 ml/head. The blood sample was inserted into the tube and the centrifuge at 3000 rpm to obtain blood serum.

Gel preparations. Assembling two glass plates with a distance between them of approximately 1 mm. The gel was consist of two layers, namely the gel as a sample holder (stacking gel) and the gel as a protein separation medium (separating gel). The gel separator was Lower Gel Buffer (LGB), T-Acryl, dd H2O, ammonium persulphate (APS), tetramethyl ethylenediamine (TEMED). The gel were dissolved together in sterile distilled water. Carefully poured into the gel layer using a micropipette and left for 10-30 minutes to form a gel. Next, the stacking gel was poured over the separating gel that had solidified while applying a comb to form a gel following its wells. Stacking gel was made from UpperBuffer, T-Acryl, APS, TEMED, and dissolved together in sterile distilled water. After forming the gel, the comb was carefully removed, and the plate was placed on the electrophoresis device.

Sample injection and running. The 150 µl hydrolyzed sample solution was added with a 1:1 buffer, then heated at 100 °C for 3 minutes. After cooling, 20 µl of the sample was pipetted, and put into an acrylamide gel well and one of the gel wells was filled with protein standard. The electrodes were installed according to the poles. Electrophoresis was run at a voltage of 130 V, 30 mA/gel for 1-2 hours. The electrophoresis with mini bio-rad process stoped if the blue marker approximately was 0.5 cm from the lower limit of the gel plate.

Treatment after running. After electrophoresis, staining was done by immersing the gel in the staining solution for 30-60 minutes by shaking it with a shaker. Removing the gel color was done by immersing the gel in a destaining solution while shaking it with a shaker until the gel became transparent so that it took documentation.

3. Results and Discussion
The results of protein profiles using Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) from blood serum of single (Figure 1) and twin (Figure 2) offspring.
3.1. Molecular Weights of Proteins in Single and Twin Offspring of ILEG
The difference of molecular weight at each replication from doe with single and twin offsprings could be seen in the Table 1.

**Table 1. Molecular Weight Differences of Protein band**

| Group | 140 | 126 | 116 | 96 | 66 | 39 | 34 | 29 | 25 | 13 |
|-------|-----|-----|-----|----|----|----|----|----|----|----|
| IBT1  | -   | -   | V   | V  | V  | V  | -  | V  | V  | V  |
| IBT2  | V   | V   | V   | V  | V  | V  | V  | V  | V  | V  |
| IBT3  | V   | V   | V   | V  | V  | V  | V  | -  | V  | V  |
| IBT4  | -   | -   | V   | V  | V  | V  | -  | V  | V  | V  |
| IBT5  | V   | V   | V   | V  | V  | V  | V  | V  | V  | V  |
| IBT6  | V   | V   | V   | V  | V  | V  | V  | -  | V  | V  |
| IBK1  | V   | V   | V   | V  | V  | V  | V  | V  | V  | V  |
| IBK2  | V   | V   | V   | V  | V  | V  | V  | V  | V  | V  |
| IBK3  | V   | V   | V   | V  | V  | V  | V  | V  | V  | V  |
| IBK4  | V   | V   | V   | V  | V  | V  | V  | V  | V  | V  |
| IBK5  | V   | V   | V   | V  | V  | V  | V  | V  | V  | V  |
| IBK6  | V   | V   | V   | V  | V  | V  | V  | V  | V  | V  |

*IBT = Female Goat with single offspring, IBK = female goat with twin offspring*

Table 1 showed that the protein weight ranged from 14.4 kDa to 200 kDa, that was 13 kDa, 25 kDa, 29 kDa, 34 kDa, 39 kDa, 46 kDa, 96 kDa, 116 kDa, 126 kDa, and 140 kDa. Female goat with twin offspring expressed almost all the protein weight compare with single offspring, that meant protein pattern fragment did not express in all-female goat with single offspring. Supported by a different study that pregnant goat was also higher at protein pattern expression than the non-pregnant goat [16].

3.2. Percentage of protein bands appearance at various molecular weights
The percentage of protein bands appearing at various molecular weights in single and twin offspring could be seen in Table 2.
Table 2. The percentage of protein bands appearing at various molecular weights

| Group | Molecular Weights (kDa) | Mean    |
|-------|------------------------|---------|
|       | 140                    | 126     | 116  | 96  | 46  | 39  | 34  | 29  | 25  | 13  |       |
| IBT   | 66.7%                  | 66.7%   | 100% | 100%| 100%| 66.7%| 100%| 100%| 100%| 100%| 90.01%|
| IBK   | 100%                   | 100%    | 100% | 100%| 100%| 100% | 100%| 83.3%| 100%| 100%| 98.33%|

IBT = Female Goat with single offspring, IBK = female goat with twin offspring

Table 2 showed that the percentage of protein bands appearance with molecular weights of 13, 25, 34, 46, 96, and 116 kDa were the same between twin and single offspring. Different protein bands were present at molecular weights of 29, 38, 126, and 140 kDa.

The appearance of protein bands at 13, 25, 34, 46, 96, and 116 kDa had various functions in the body's metabolism. The molecular weight of 13 kDa was a relationship with β lactalbumin. The molecular weight of 25 kDa was C1q protein [17-19]. The molecular weight of 30-100 kDa was albumin rich [20] that functioned to maintain the balance of osmotic pressure, protein reserves, and binding [21].

The molecular weight of 29 kDa molecular weight showed 83.6% similarity between single and twin offspring. The protein was immunoglobulin G (IgG) that functioned in the body's defense and immune system [20-21]. The molecular weight of 39 kDa showed a percentage of 66.7% in single offspring and 100% in twin offspring. Twin offspring was 33.3% higher than single offspring. The molecular weight of 39 kDa was assumed to be a protein encoded by the SRY gene. SRY genes were concentrated in areas of High Mobility Groups (HMG).

High molecular weights 126 and 140 kDa was a percentage of 66.7% in single offsprings and 100% in twin offsprings (33.3% higher in twin offsprings than single offsprings). The proteins were suspected in the process of ovulation. Female goats with twin offsprings got ovulated eggs more than a single offspring caused a higher inflammatory reaction. Supported by the opinion that the high molecular weight fraction contained component of defense reaction to keep the body from being uncomfortable [22].

3.3. Protein band thickness

The results showed that various protein bands thickness at fragment 44-94 kDa; a female goat with twin offspring was a thicker protein band than single offspring (Figure 3). Figure 3a described the protein band thickness of female with single offspring, and 3b figured for twin offspring.

Figure 3. Protein band thickness (a) IBT (single offspring) and (b) IBK (twin offsprings)

Figure 3 showed the specific protein fragment at 44-94 kDa suspected played a role in folliculogenesis [23]. Folliculogenesis is the process of follicle formation in ovarian of female reproduction. It must be present to support embryo development [24], and the protein fragment of 52 kDa was a candidate for pregnancy early indicator in goats [16].

4. Conclusions

Serum protein profile between female ILEG of single and twin offspring had ten bands formed with molecular weights ranging from 13-140 kDa. The protein band appearance of female ILEG with twin offspring was 8.32% higher than single offspring (98.33% vs. 90.01%). Protein band thickness at 44-

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*Note:* The provided text appears to be a continuation of a scientific paper discussing protein band analysis in female goat populations, including the methodology, observed patterns, and implications of the findings. The text is rich in scientific detail and context, possibly pertaining to a research study aimed at understanding protein expression and its role in physiological processes. The table and figures mentioned are integral to illustrating the statistical and visual data collected during the experiments. The concluding section reflects on the broader applications of the findings, potentially informing future research directions in goat reproduction and disease resistance studies.
94 kDa increased expression in the ILEG of twin offspring instead of single offspring. The research recommends molecular protein weight at 44-94 kDa as a candidate to an early detect female goat with twin offspring.

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References
[1] Mudawamah ID, Retnaningtyas MF, Wadjdi, Badriyah, Susilowati S, Aulanni’am and Ciptadi G 2014 Jurnal Kedokteran Hewan 8(2) 138-141
[2] Mudawamah ID, Ratnaningtyas, Usman A, Fadli MZ and Ciptadi G 2016 Asian Jr. Of Microbiol. Biotech. Env. Sci. 20 S182-S187
[3] Amnate AA, Mohammed QS, Mahdi ZM, Mahdi AJ, Jaffar HM, Sammen MM and Hamd RA, Al-Anbar 2016 J. Vet. Sci. 9(2) 94-99
[4] Mudawamah M, Fadli MZ, Ciptadi G, Ali U and Putri GR 2019 AIP Conference Proceedings 050013 1-4
[5] Mudawamah M, Ratnaningtyas ID, Fadli MZ and Ciptadi C 2019 ICAMBBIE 2018. IOP Conf. Series: Journal of Physics: Conf. Series 1146 1-5
[6] Polley S, De S, Batabyal S, Kaushik R, Yadav P, Arora JS, Chattopadhyay S, Pan S, Brahma B, Datta TK, Goeswami LS 2009 Small Ruminant Research 85(2-3) 122-129.
[7] Mishra C, Rout M, Mishra MP, Sahoo SS, Nayak G and Patra RC 2017 Explor Anim Med Res. 7(2) 132-141
[8] Wu XH, Mu XK, Lu JY, Wang Y and Zi XD 2014 Journal of Applied Animal Research 42(4) 414-419.
[9] Zheng J, Raza SHA, Zi XD, Lu JY 2011 Genetics and Molecular Research 17(4) 1-11
[10] Sarma L, Nahar deka N, Zaman G, Aziz A, Das A, Akhtar A, Upadhyay S and Borkoliita L 2019 Journal of Entomology and Zoology Studies 7(2) 34-37.
[11] Thomas N, Venkatachalapathy T, Aravindakshan T, Raghavan KC 2016 Animal Reproduction Science 167 8-15.
[12] Zhang C, Liu Y, Huang K, Zeng W, Xu D, Wen Q and Yang L 2011 Genetics and Molecular Biology 34(1) 49-55.
[13] Munoz G, Ovilo C, Estelle J, Silio L, Fernandez A and Rodriguez C 2006 Genet. Sel. Evol. 39 195-206.
[14] Hofmann L and Paleczewski K 2017 Methods Mol Biol 1271 3-18
[15] Halder A, Pal SK, Chakraborty S, Hazorika M, Pan S, Majumdar D, Biswas CK, Patra A, Mirmahmoudi R and Prakash BS 2013 Animal Reprod Sci. 140(1-2) 54-61.
[16] Inyawilert W, Piarked A, Joemplang P, Tatsapong P, Tiantong A 2019 Advances in Animal and Veterinary Sciences 7(12) 1049-1053.
[17] Moreno-Indias I, Dodds AD, Argüello A, Castro N and Sim RB 2012 BMC Veterinary Research 8 91-99.
[18] Ambrose DJ, Radke B, Pitney PA, Goonerwardene LA 2007 Can Vet Journal 49 931-935.
[19] Anderson NL, Aderson NJ 2002 Molecular & Cellular Proteomic 1(11) 845-867.
[20] Morais C, Westhuyzen J, Metharom P and Healy H (2005) Nephrol Dial Transplant 20 50-58.
[21] da Costa WKA, de Souza EK, o-Filho EMB, Vasconcelos KG, Santi-Gadelha T, Alberto C 2014 PLOS ONE 9(3) e93361 1-8.
[22] Leeman M, Choi J, Hansson S, Storm MU and Nilsson L 2018 Analytical and Bioanalytical Chemistry 410 4867–4873
[23] Schuller AGP, Lindenbergh-Kortleve, Pache TD, Zwarthoff EC, Fauser BCJM, Drop SLS 1993
regulatory peptides 48(1-2) 157-163.

[24] Marta R, Knap S, Jankowski M, Jeseta M, Bukowska D, Antosik P, Nowicki M, Zabel M, Kempisty B, Jaskowski JM 2018 Medical Journal of Cell Biology 2018 33-38.