IN- SILICO CHARACTERIZATION OF OVINE PROGESTERONE RECEPTOR

***Musa, I.S., Gambo, D. and Abdulrahman, N.**
Department of Animal Science, Nasarawa State University, Keffi, Nigeria
*Corresponding email: dribmazara@yahoo.com; +2348036140045

ABSTRACT
The objective of this study was to determine the molecular structure of Ovine progesterone receptor using computational methods. The protein sequences of ovine progesterone with accession number DQ318952 was retrieved from the GenBank (NCBI) for molecular analysis. Protparam tool was used for determination of physicochemical parameters of the protein. The secondary structure of ovine progesterone receptor was predicted using GOR 4 server. The result indicates a total of 350 amino acid residues with Leucine as most predominant (14.6 %) and methionine having the least percentage (0.9 %). The protein had a net positive charge. The predicted secondary structure indicated 40.29 % alpha helix, 15.7 % extended strand and 44.0 % random coil. The predicted instability index of 46. 95 % showed that the ovine progesterone receptor is probably unstable. Further studies on identifying polymorphism in ovine progesterone receptor will provide more highlight in genetic factors of luteal failure in the ovine species.
Keywords: Molecular, Analysis, Ovine progesterone receptor, In silico

INTRODUCTION

Progesterone receptor is a cellular protein activated by progesterone. The steroid hormones and their regulators are involved in the regulation of gene expression and affects cellular proliferation and differentiation (Ryan *et al*., 2011). Progesterone is required for establishment and maintenance of pregnancy in all mammals. Its effects are mediated through progesterone receptors. Progesterone plays a critical role in maternal support and conceptus survival and development. It is a key coordinator of female reproduction playing a direct role in ovulation, uterine growth, implantation and mammary gland development (Person *et al*., 1981). Progesterone receptor is a member of the steroid receptor super family and function as a progesterone modulated transcription factor in most species (Lydonet *et al*., 2005). Endocrine factors are reported to play a role in early embryonic death (Mamo *et al*., 2012). Early embryonic mortality accounts for majority of reproductive failure in farm animals. It is a significant cause of pregnancy loss and repeat breeding in domestic animals. Increased understanding of hormonal factors involved in pregnancy establishment will provide better insight into the causes of infertility and recurrent early pregnancy loss in domestic animals. The main objective of this study was to evaluate the molecular characteristics of ovine progesterone receptor using computational methods.

MATERIALS AND METHODS

Protein sequence of Ovine progesterone receptor with accession number DQ318952.1 was retrieved from the GenBank for molecular analysis.

Determination of Physical and chemical properties of the Ovine progesterone receptor

ProtParam Tool was used for the computation of various physical and chemical properties of the Ovine progesterone receptor using protein sequences. The computational parameters include molecular weight, theoretical pI (isoelectric point), amino acid composition, atomic composition, extinction coefficient, estimated half-life, instability index, aliphatic index and grand average of hydropathicity (GRAVY) (Gasteiger, 2005). Protparam is a tool which allows the computation of various physical and chemical parameters for a given protein stored in Swiss –Prot or for a user entered protein sequence.

Prediction of secondary structure of ovine progesterone receptor.

The amino acid sequences of Ovine progesterone receptor were further subjected to secondary structure prediction using GOR 4 server for prediction of secondary protein structures. The method is based on probability parameter derived from studies of known protein tertiary structure solved by x-ray crystallography. It also takes into account the condition probability of the amino acid to form a secondary structure (Kouza et al., 2017).

Prediction of 3D structure

The Phyre2 server was used to predict the 3D structure of ovine progesterone receptor. The server predicts the three-dimensional structure of a protein sequence using the principles and techniques of homology modelling (Kelley and Sternberg, 2009).

RESULTS

The amino acid composition of ovine progesterone receptor is presented in Table I. The result indicates 350 amino acid residues, leucine is the most dominant amino acid (14 %), while methionine has the least percentage (0.9 %). The protein structure consists of 20 different amino acids.

TABLE I: Amino acid composition of ovine progesterone receptor

Amino acid	Number of residue	Percentage (%)
Ala (A)	37	10.6%
Arg (R)	16	4.6%
Asn (N)	4	1.1%
Asp (D)	11	3.1%
Cys (C)	6	1.7%
Gln (Q)	18	5.1%
Glu (E)	11	3.1%
Gly (G)	14	4.0%
His (H)	17	4.9%
Ile (I)	11	3.1%
Leu (L)	51	14.6%
Lys (K)	8	2.3%
Met (M)	3	0.9%
Phe (F)	29	8.3%
Pro (P)	25	7.1%
Ser (S)	20	5.7%
Thr (T)	16	4.6%
Trp (W)	9	2.6%
Tyr (Y)	16	4.6%
Val (V)	28	8.0%

The physicochemical parameters of ovine progesterone receptor is shown in Table 2. The parameters evaluated were molecular weight, theoretical pi, extinction coefficient, estimated half-life, instability index, aliphatic index and grand average hydropathacity.

TABLE 2: Physicochemical parameters of ovine progesterone receptor

Parameters	Values
No. of amino acid residues	350
Molecular weight	3989149
No. of negatively charged residues	22
No. positively charged residues	24
Extinction coefficient	73715
Instability index	4695
Aliphatic index	102.86
Grand average hydropthicity	0.332

The secondary structure of ovine progesterone receptor predicted using GOR 4 server is shown in Table 3. The result indicated 40.2 % alpha helix, 15.71% extended strand and 44.0 % random coil.

TABLE 3: Predicted secondary structures of ovine progesterone receptor

Structure	Percentage (%)
Alpha helix	40.29
Extended strand	15.1
Random coil	44.00

DISCUSSION

The amino acid composition of ovine progesterone receptor is shown in table 1. The results indicate that ovine progesterone receptor has a total of 350 amino acid residues; leucine is the predominant amino acid (14.6%) while methionine has the least percentage (0.9%). The physico chemical properties of ovine progesterone receptor is presented in table 2. The analysis showed a total of 22 negatively charged residues and 24 positively charged residue, indicating a net positive charge. The amino acid sequences determine the higher structural levels of proteins (secondary, tertiary and quaternary) and specify their biological properties. (Branden and Tooze 1991). Different amino acids play important roles in formation of third structure of protein, some amino acids such as alanine, glutamic acid and leucine trend to participate in α-helix and some others trend to take part in β-sheet. Variation in amino acid composition tend to change the structure of the obtained protein leading to the change in conformation of third structure of the protein and its function. Many disease-related effects of polymorphisms involve effects on protein structure and/or function. Thus, accurate predictions of the effect of polymorphisms on the structure and function of protein gene products are essential (Wang and Moult, 2001). Instability index determined the stability of a protein in a test tube, values less than 40 indicate that protein is probably stable whereas values more than 40 indicate probably unstable protein (Guruprasad et al., 1990). Predicted instability index of 46.95 indicate that ovine progesterone receptor is probably unstable. The aliphatic index of a protein is defined as the relative volume occupied by aliphatic side chains (alanine, valine, isoleucine, and leucine). It may be regarded as a positive factor for the increase of thermostability of globular proteins (John, 2005). The extinction coefficient indicates how much light a protein absorbs at a certain wavelength. The half-life is a prediction of the time it takes for half of the amount of protein in a cell to disappear after its synthesis in the cell. The predicted secondary structure of ovine progesterone receptor indicates 40.24 % alpha helix, 15.71% extended strand and 44.0% random coil as shown in table 3. The predicted 3 D structure of the protein is presented in figure 1. The shape of a protein is critical to its function because it determines whether the protein can interact with other molecules.

REFERENCES

Brandin, C. and Tonze, J. (1991): Introduction to Protein structure. New York, Garland Publishing. Guruprasad, K., Reddy, B. and Pondit, M. W. .(1990): Correlation between stability of a protein and its dipeptide composition : a novel approach for predicting in vivo stability of a protein from its primary sequence. Protein engineering, 4(2): 155-61

John, M.W. (2005): The Proteomics Protocols Hand book .Hormona Press. 571-607

Kouza, M., Feragli, E., Kolinsi, A.. and Klokowski, A.. (2017): The GOR method of secondry Protein structure prediction and its application as a protein aggregation prediction tool Methods. Mol. Biol. 1484 : 7 – 24

Lydon, J. P. Demayo, F. J., Funck, C. R., Mani, S. K., Hughs, A. R., Montgomary, C. A., Shymala, G., Conneely, O. M. and O'Malley, B. W. (2005): Mice lacking progesterone receptor exhibit pleotropic reproductive abnormalilities . *Genes Dev* 9, 2266-2278

Mamo, S., Mehta, J. P., Forde, M. Gettigan, P. and Lonagan, P. (2012): Conceptus endometrium crosstalk during maternal recognition of pregnancy in cattle. Biological Reproduction, 87 .

Parsons, B., McGinis, M. Y. and McEwen, B. S. (1981): Sequential Inhibition of Progesterone: effects on sexual receptivity and associated changes in Brain cytosol progestine binding in the female rat. *Brain Resources.* 221: 149-160.

Ryan, J., Grennfield, D., Marin, R., Preston, C., Roman, B., Jensen, S., Pargett, D., Birch, J., Mikrulski, C., Dousetta, G. and Scholin, C. (2011): Harmful Phytoplankton Ecology Studies using an Autonomous Molecular Analytical and Ocean Observing network. *Limnology and Oceanography,* 56(**4):** 1255 1272.

Wang, Z. and Moult, J. (2001) : Salps Protein Structure and Disease. *Human Mutation*, 17:263-270