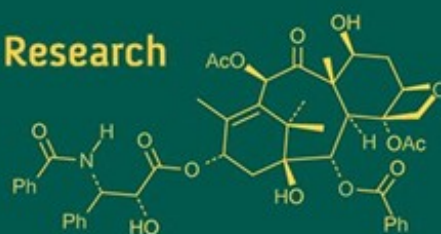


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Sex-associated differences in circulating protein concentrations in Andamani goats

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ABSTRACT

Sex-associated variation in circulating proteins may provide important insight into the physiological and molecular regulation of reproduction in goats. The present study evaluated serum concentrations of four candidate proteins insulin-like growth factor 1 receptor (IGF1R), SET domain bifurcated histone lysine methyltransferase 2 (SETDB2), fibroblast growth factor receptor 1 (FGFR1), and growth differentiation factor 9 (GDF9) in Andamani goats to characterize sex-associated protein profiles. The study included 158 non-gravid adult does and 15 bucks, with protein concentrations quantified using goat-specific sandwich ELISA kits. Differences between sexes were evaluated using the Wilcoxon rank-sum test followed by Benjamini–Hochberg false discovery rate (FDR) correction. Mean concentrations of all four proteins were numerically higher in males than females, with the largest differences observed for FGFR1 and SETDB2. Although FGFR1 and SETDB2 showed nominal sex-associated differences ($P = 0.025$ and 0.045 , respectively), neither remained significant after FDR correction ($FDR = 0.090$), while IGF1R and GDF9 showed no significant differences. Overall, no statistically significant sex effect was detected for the four candidate proteins after correction for multiple comparisons. The numerical differences, particularly for FGFR1 and SETDB2, suggest potential sex-associated variation warranting further investigation. However, the smaller male sample size necessitates cautious interpretation and validation in larger, more balanced populations.

Keywords: Goat prolificacy, IGF1R, SETDB2, GDF9, FGFR1, Andaman goat, ELISA, Reproductive biomarkers

Introduction

Goats (*Capra hircus*) constitute an important livestock resource in tropical and subtropical regions because of their adaptability to diverse environmental conditions, ability to utilize relatively low-quality feed resources, and contribution to meat production and rural livelihoods (Inbaraj *et al.*, 2018) [1]. In the Andaman and Nicobar Islands of India, goats are maintained under both organized and farmer-managed production systems and comprise locally adapted Andamani goats and their admixed populations. Improving reproductive efficiency in these populations is important for enhancing kid production and the overall productivity of meat-oriented goat production systems (Ahlawat *et al.*, 2015) [2].

Sex is an important biological source of variation in circulating protein profiles, as males and females differ in their endocrine milieu, reproductive physiology, metabolism, growth patterns, and regulation of protein synthesis and turnover. Sex steroids and gonadotropic hormones can influence gene transcription, protein synthesis, secretion, and degradation, while sex-specific reproductive functions may further modify proteins involved in growth-factor signalling, gonadal development, gametogenesis, metabolism, and cellular regulation. Consequently, characterization of sex-associated differences in circulating proteins can provide useful baseline information for distinguishing general physiological variation between males and females from molecular changes associated specifically with reproductive performance.

Several growth factors and signalling proteins involved in reproductive regulation are expressed in both sexes but may exhibit sex-dependent patterns of regulation. The insulin-like growth factor (IGF) system, including insulin-like growth factor 1 receptor (IGF1R), is involved in cellular growth, differentiation, metabolism, and gonadal function. IGF1R-mediated signalling has also been implicated in follicular development, steroidogenesis, cell proliferation, and survival (Wang *et al.*, 2011; Thomas *et al.*, 2016; Mahmoudi *et al.*, 2019; Wang *et al.*, 2021; Wei *et al.*, 2025) [3, 4, 5, 6, 7]. Thus, variation in circulating IGF1R concentration between sexes may reflect differences in the physiological regulation of the IGF signalling system, although circulating receptor concentrations do not directly represent receptor expression or activation within reproductive tissues.

Growth differentiation factor 9 (GDF9), a member of the transforming growth factor- β superfamily, is an important regulator of ovarian follicular development and oocyte–somatic cell communication. Its established

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involvement in female reproductive processes makes GDF9 a biologically relevant candidate for investigating reproductive variation. However, comparison between males and females may also provide useful information regarding the extent to which circulating GDF9 concentrations exhibit sex-associated variation, thereby facilitating interpretation of its potential relationship with female reproductive phenotype.

The fibroblast growth factor (FGF) signalling system represents another important regulatory pathway involved in cellular proliferation, differentiation, tissue development, and reproductive function. These biological effects are mediated through fibroblast growth factor receptors, including FGFR1, which participates in intracellular signalling processes associated with growth and development and has been implicated in gonadal and reproductive physiology (Brooks *et al.*, 2012; Haugsten *et al.*, 2010; Meng *et al.*, 2014; Fulin *et al.*, 2023) [8, 9, 10, 11]. Evaluation of circulating FGFR1 in both sexes may therefore provide additional insight into sex-associated differences in growth-factor-related regulatory mechanisms.

In addition to growth-factor signalling, epigenetic mechanisms may contribute to sex-specific regulation of reproductive processes. SET domain bifurcated histone lysine methyltransferase 2 (SETDB2) is involved in epigenetic regulation through histone H3 lysine 9 methylation and consequent modulation of transcriptional activity. Epigenetic regulatory mechanisms have been implicated in gonadal development and reproductive function, suggesting that SETDB2 may represent a candidate protein of interest in reproductive biology (Falandry *et al.*, 2010; Maekawa *et al.*, 2016) [12, 13]. Differences in circulating SETDB2 concentrations between males and females could therefore indicate sex-associated variation in this regulatory protein; however, such differences should not be interpreted as direct evidence of altered epigenetic activity without corresponding tissue-level and functional investigations.

Collectively, the involvement of GDF9, IGF1R, FGFR1, and SETDB2 in reproductive, growth-factor, and regulatory processes provides a biological basis for investigating their circulating concentrations in relation to sex. Importantly, differences in serum protein concentrations between males and females may reflect the combined influence of endocrine, gonadal, metabolic, and physiological factors and should not necessarily be interpreted as direct indicators of reproductive capacity.

Despite the importance of goats in the Andaman and Nicobar Islands, information regarding sex-associated variation in circulating candidate proteins in Andamani goats remains limited. Establishing such baseline information is important for interpreting subsequent associations between candidate proteins and female reproductive traits, including fecundity. Therefore, the present study evaluated the serum concentrations of four candidate proteins SETDB2, FGFR1, GDF9, and IGF1R in male and female Andamani goats using goat-specific sandwich ELISA, with the objective of characterizing sex-associated variation and establishing baseline protein profiles for subsequent interpretation of their potential relevance to reproductive performance.

Materials and Methods

The present study was conducted on Andamani goats maintained in the Andaman and Nicobar Islands, India. A total of 158 non-gravid adult female Andamani goats and 15 adult bucks were included in the study to evaluate sex-related differences in the selected protein biomarkers and to establish sex-specific baseline data.

Blood samples were collected from the jugular vein of the selected animals and stored in clot-activated vials for protein analysis. Following clot formation, samples were centrifuged at 1,000-2,000 × g for 10 minutes in a refrigerated centrifuge, and the separated serum was transferred into clean tubes and stored at -80 °C until analysis. Protein estimation was carried out using diluted serum samples with goat-specific Sandwich ELISA kits (Progenbiolabs) for the target proteins: SETDB2 (Goat SETDB2 ELISA Kit, PT-EG6149), FGFR1 (Goat FGFR1 ELISA Kit, PT-EG6629), GDF9 (Goat GDF9 ELISA Kit, PT-EG6633), and IGF1R (Goat IGF1R ELISA Kit, PT-EG6623).

The absorbance was measured at 450 nm within 15 minutes of color development using a microplate reader (LisaScan® EM, Erba® Mannheim).

Data collection and statistical analysis. All statistical analyses were performed using R software (version X.X.X). Protein concentrations of IGF1R, SETDB2, FGFR1, and GDF9 were expressed as mean ± standard error of the mean (SEM). Normality of the data was assessed using the Shapiro–Wilk test. As the data were not normally distributed, differences in protein concentrations between male and female groups were evaluated using the Wilcoxon rank-sum test.

To account for multiple comparisons across the four proteins, *P*-values were adjusted using the Benjamini–Hochberg false discovery rate (FDR) method (Fig. 3). An FDR-adjusted *P*-value < 0.05 was considered statistically significant. Protein distributions between sexes were visualized using box-and-whisker plots (Fig. 2).

Result and Discussion

The study reveals that physiological differences between males and females encompass much more than their reproductive functions, influencing a wide range of anatomical, hormonal, metabolic, and molecular processes throughout the body (Miller *et al.*, 2004) [14].

In the present study the male group showed higher mean concentrations of IGF1R, SETDB2, FGFR1 and GDF9 compared with females (Table 1, Fig. 1). The substantially smaller number of males (*n* = 15) compared with females (*n* = 158) limits the precision and statistical power of the sex comparison. Therefore, the observed sex-associated patterns should be considered preliminary and require validation in larger, more balanced populations. Because the data were non-normally distributed, the Wilcoxon rank-sum test (Fig. 2) was done, followed by Benjamini–Hochberg FDR correction (Fig. 3) for the four comparisons. Large-scale proteomic studies also demonstrate that circulating protein abundance can differ substantially between sexes, although sex differences in protein concentrations do not necessarily imply fundamentally different genetic regulation (Koprulu *et al.*, 2025) [15].

IGF1R showed the smallest apparent sex difference, with females having a mean concentration of 14.0 ± 0.60 and males 15.2 ± 2.29. Importantly, the median was identical in both groups, suggesting very similar central tendencies (Table 1). The sex difference was clearly non-significant (raw *P* = 0.746; FDR = 0.746) in Fig 3.

IGF1R showed the smallest sex-associated difference, with almost identical median concentrations in males and females. The IGF signalling system has broad functions in both male and female reproductive physiology, including gonadal development, steroidogenesis, germ-cell regulation and follicular function. Therefore, the comparable circulating IGF1R concentrations may reflect its fundamental physiological importance in both sexes rather than a sex-specific function. Importantly, circulating IGF1R concentration should not be interpreted as a direct measure of receptor expression or activation within reproductive tissues.

The comparable IGF1R concentrations observed between sexes may reflect the fundamental involvement of IGF1R-mediated signalling in reproductive processes of both males and females, including gonadal development, steroidogenesis, follicular function and germ-cell regulation (Neirijnck *et al.*, 2019) [16]. IGF ligands and their receptors play important roles in gonadal development, sex determination and reproductive function, with locally produced IGFs acting through autocrine and paracrine mechanisms within reproductive tissues (Neirijnck *et al.*, 2019) [16]. Insulin/IGF signalling is particularly important for male gonadal differentiation, as disruption of insulin receptor-family signalling, including IGF1R, has been shown to impair testicular differentiation and male sex determination in experimental models (Nef *et al.*, 2003) [17]. Thus, the present results suggest that although IGF1R is relevant to reproductive physiology, its circulating concentration did not exhibit a detectable sex-associated difference in this population.

SETDB2 showed a more pronounced numerical difference (Table 1), increasing from 194 ± 8.19 in females to 259 ± 32.0 in males, with

corresponding medians of 172 and 228. The raw comparison reached nominal significance ($P = 0.045$), but this association did not persist after correction for multiple testing ($FDR = 0.090$). Therefore, the result represents a suggestive sex-associated pattern rather than statistically robust evidence of a sex effect.

SETDB2 is an epigenetic regulator with potential relevance to germ-cell differentiation. Notably, *Setdb2* transcripts have been identified in germ cells of both embryonic ovaries and testes, and SETDB2 protein has been localized to germ cells during the transition from mitosis to meiosis (Hogarth *et al.*, 2011) [18]. These observations provide biological support for a role of SETDB2 in reproductive tissues of both sexes. However, current evidence does not establish that circulating SETDB2 should normally be higher in males. Consequently, the higher male concentration observed here is an interesting preliminary finding that warrants confirmation in a larger and more balanced population.

FGFR1 demonstrated one of the strongest numerical sex differences (Table 1). Females had a mean concentration of 64.8 ± 2.02 , compared with 84.8 ± 8.73 in males, while the respective medians were 63.8 and 93.0. The raw P -value was significant ($p = 0.025$), but the association was attenuated after multiple-testing correction ($FDR = 0.090$). This is therefore appropriately described as a nominal sex-associated difference (Fig. 1) that did not withstand FDR correction (Fig. 3).

There is substantial biological evidence linking FGFR1 signalling with male reproductive function. FGFR1 is expressed in testicular germ cells and Leydig cells, while FGF/FGFR signalling contributes to testicular development, spermatogenesis, spermiogenesis and sperm maturation. Disruption of FGFR1 signalling during spermiogenesis has been associated with reduced daily sperm production and impaired sperm capacitation, supporting its role in normal male reproductive function (Cotton *et al.*, 2008) [19]. These findings provide a plausible biological context for the higher FGFR1 concentrations observed in males. Nevertheless, evidence from reproductive tissues should not be interpreted as direct proof that circulating FGFR1 concentrations are physiologically higher in males, particularly because the present FDR-adjusted result was non-significant.

GDF9 concentrations were also numerically higher in males (26.1 ± 5.00) than females (18.7 ± 1.31), and the median increased from 11.7 in females to 19.1 in males (Table 1). However, the difference was not statistically significant either before ($P = 0.093$) or after FDR correction (Fig. 3). The broad female range (0.691–71.0) also indicates considerable inter-individual variation.

Although GDF9 is best known as an oocyte-derived growth factor regulating granulosa-cell proliferation and follicular development, it is not restricted to female reproduction. Nicholls *et al.* (2009) [20] demonstrated GDF9 expression in male germ cells, particularly round spermatids, and showed that GDF9 can regulate Sertoli-cell functions, including inhibin B production. This provides a strong biological explanation for the presence of GDF9 in males. However, it does not demonstrate that circulating GDF9 should be higher in males, and

therefore the numerical difference observed in the present study requires further investigation.

Overall, the results (Table 1) reveal an interesting hierarchy of apparent sex-associated differences: FGFR1 and SETDB2 showed the strongest evidence, GDF9 showed a weaker tendency, and IGF1R showed virtually no evidence of separation between sexes (Fig. 1). SETDB2 and FGFR1 initially reached nominal significance, but neither remained significant after controlling for multiple comparisons. Thus, the FDR-adjusted results (Fig. 3) provide no statistically significant evidence of a sex effect for any of the four proteins.

Biological plausibility nevertheless exists for the observed patterns. FGFR1 has established functions in testicular development and spermatogenesis, GDF9 is expressed in male germ cells and regulates Sertoli-cell function, SETDB2 is expressed in developing germ cells of both sexes, and the IGF/IGF1R system contributes broadly to male and female reproductive development. The observed numerical differences provide preliminary evidence of possible sex-associated variation in circulating FGFR1 and SETDB2 concentrations, but these findings require confirmation in larger and more balanced populations.

Conclusion

The four candidate proteins showed numerically higher circulating concentrations in males than females, with the greatest differences observed for FGFR1 and SETDB2. Although these two proteins showed nominal sex-associated differences, neither remained significant following FDR correction, while IGF1R and GDF9 were non-significant in both unadjusted and adjusted analyses. The observed trends are biologically plausible given the established involvement of FGFR1, GDF9, SETDB2 and IGF1R-associated pathways in gonadal development, germ-cell differentiation and reproductive signalling (Cotton *et al.*, 2008; Nicholls *et al.*, 2009; Neirijnck *et al.*, 2019) [19, 20, 16]. The findings provide preliminary information on sex-associated variation in circulating concentrations of the four candidate proteins and warrant validation in larger populations.

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Conflict of Interest

The authors declare no conflict of interest.

Factor	Mean $\pm$ SEM (n)			
Sex	IGF1R	SETDB2	FGF1R	GDF9
Female	14.06 ± 0.65 (158)	194.18 ± 8.19 (151) ^b	64.85 ± 2.04 (152) ^b	18.65 ± 1.32 (148)
Male	15.18 ± 2.10 (15)	258.67 ± 32.00 (13) ^{a*} (wt)	84.81 ± 7.59 (11) ^{a**}	26.14 ± 4.46 (13)

* $p \leq 0.05$, ** $p \leq 0.01$, wt = welch test

(continued on next page)

Table 1. Sex-associated differences in circulating concentrations of the selected proteins in Andamani Goats.

Protein	Sex	n	Mean ± SEM	SD	Median	Min	Max
IGF1R	Female	155	14.0 ± 0.60	7.47	12.8	1.58	54.0
IGF1R	Male	15	15.2 ± 2.29	8.87	12.8	5.09	38.3
SETDB2	Female	151	194.0 ± 8.19	101.0	172.0	13.4	548.0
SETDB2	Male	13	259.0 ± 32.0	115.0	228.0	91.5	458.0
FGF1R	Female	152	64.8 ± 2.02	24.9	63.8	10.3	156.0
FGF1R	Male	11	84.8 ± 8.73	29.0	93.0	37.4	121.0
GDF9	Female	148	18.7 ± 1.31	15.9	11.7	0.691	71.0
GDF9	Male	13	26.1 ± 5.00	18.0	19.1	6.21	62.6

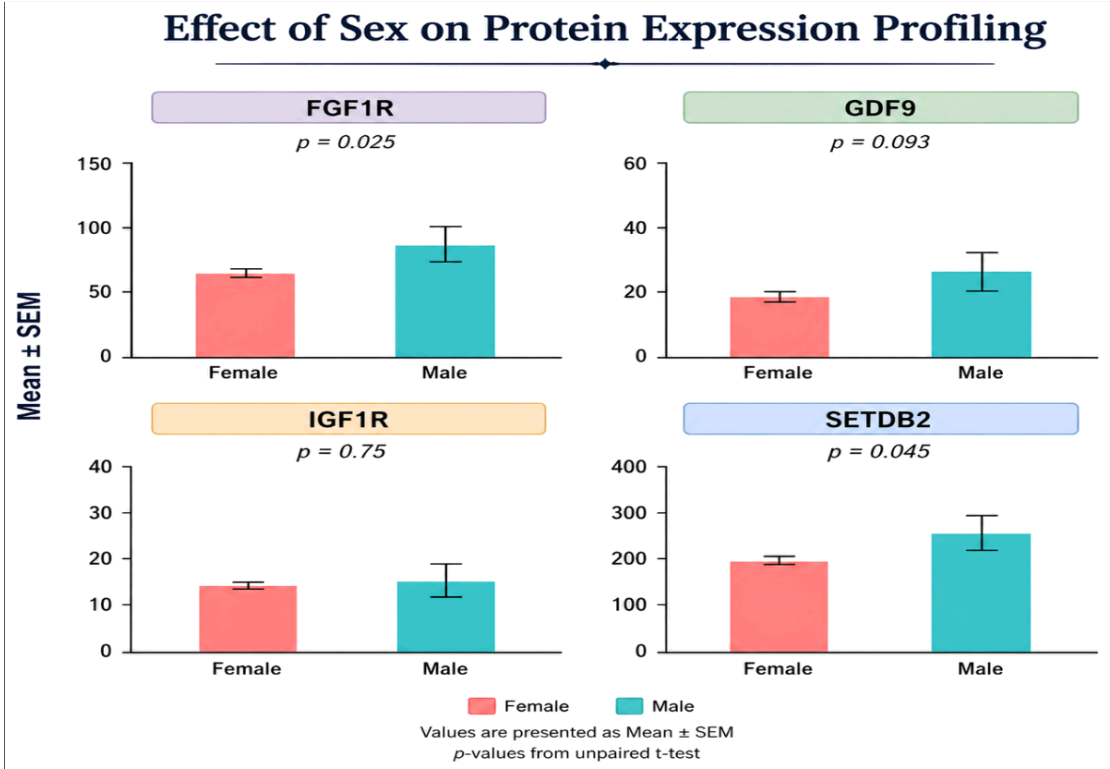


Table 2. Descriptive Statistic

Figure 1. Sex-associated differences in circulating protein concentrations

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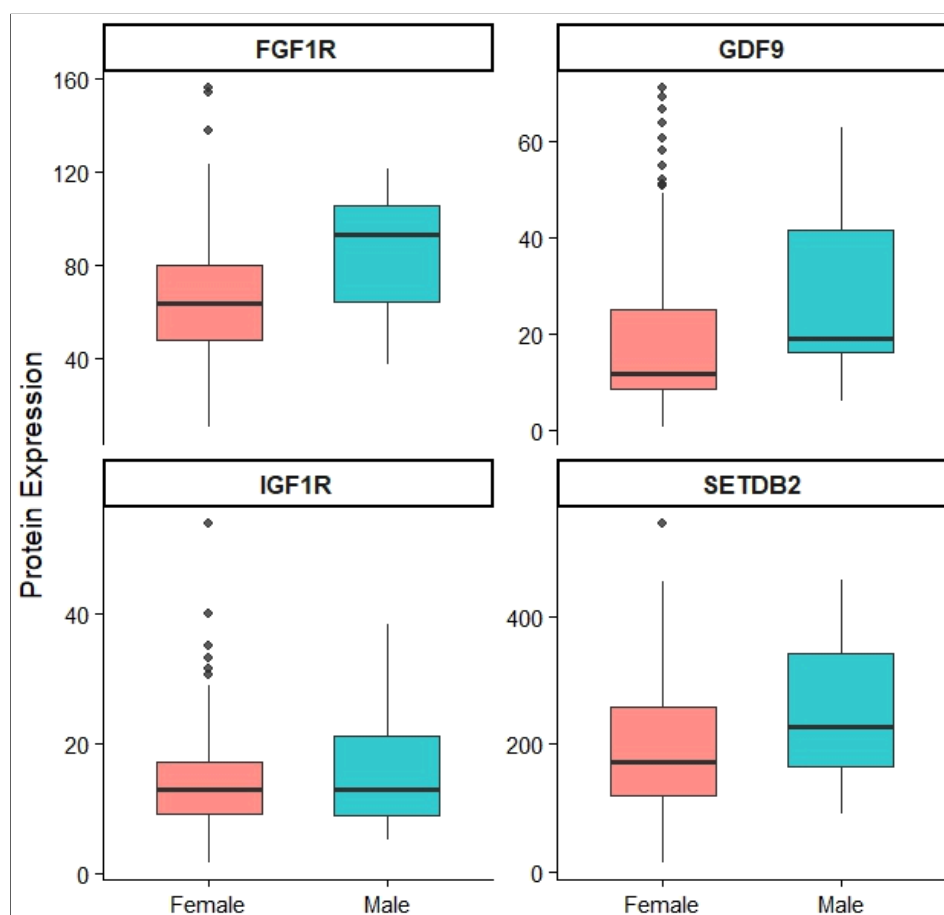


Figure 2. Distribution of circulating protein concentrations according to sex

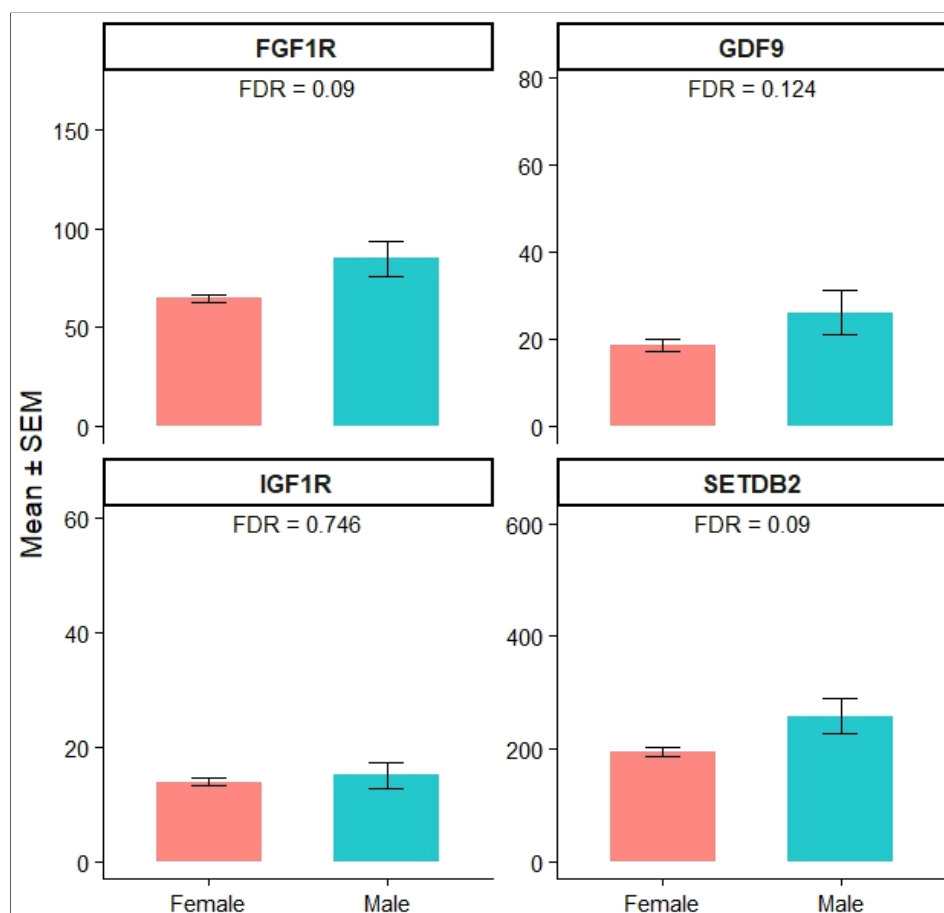


Figure 3. Benjamini-Hochberg FDR-adjusted comparisons on protein concentrations

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