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Research Article

VARIATION IN SEMEN QUALITY BECAUSE OF SOYBEAN ADDED NUTRITION AMONG HEALTHY MEN AT SASARAM, BIHAR, INDIA

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A B S T R A C T

Alarming decreases in world trends of semen quality have been ascertained. Moreover, regional variations in semen quality have also been reported. Because comprehensive research on male reproductive function in Bihar is lacking, the aim of this study is to characterize infertility in males of Sasaram at a regional level through semen parameters. Cross-sectional data were collected from males of age group 20-59 years visiting at Kumar Janch Ghar, Sasaram from 2018 to 2019 (n=2117). We tend to evaluate the effect of soybean added nutrition on changes in conventional semen parameters and therefore the potential mechanisms involved. Measures of central tendency, simple and pair-wise correlation, multivariate analysis and two-way ANOVA were applied during this study. The work inferred that the inclusion of soybean in the diet in a very routine north Indian diet significantly ($P<0.05$) declined the total sperm count, sperm viscosity, sperm density, sperm viability but increase sperm motility. Therefore, we propose that semen parameters could be related to the inclusion of soybean in the diet of men. These findings have to be complied to be more investigated in the general population and large sample cohort studies.

INTRODUCTION

Concerns over a world decline in sperm quality have attracted the attention of the scientific community and public alike, pointing to changes in modifiable lifestyle factors because of the main causes [1, 2]. It is estimated that 15% of couples worldwide have issues conceiving and malefactors are responsible for 40–50% of those cases [3, 4, 5, 6]. Time and over again, numerous studies have been published, supporting a compromise in sperm quality or dismissing a similar [7]. More recently, some systematic reviews of observational studies and randomized controlled trials found out that male adherence to a healthy diet rich in ω- 3 antioxidants, carnitines and folate could improve semen quality and fecundity [8, 9].

Soy products, processed meats, trans fats, pesticides, fish etc. are foods that decrease sperm health. Soybean and soy products decrease sperm quality because of the high content of a type of phytoestrogen known as isoflavones. Theoretically, this exposure to high levels of phytoestrogen in men could alter the hypothalamic-pituitary-gonadal axis [10]. Many studies on animals have shown that such a hormonal effect could also be vital and decrease fertility. In recent years, phytoestrogens are popular as dietary supplements and widely consumed for disease prevention and adjunctive therapy by the general population, because of their potential health benefits on cancer [11]. However, for many populations, especially in Asians, the daily intake of phytoestrogens is much higher than most of other environmental endocrine disrupter chemicals and therefore the endogenous estrogens [12]. There are controversies associated with the reproductive health of men related to phytoestrogens [9, 13]. Phytoestrogens could also be considered as exposure biomarkers. Furthermore, Asians possess a greater ability to convert soya to a non-steroidal oestrogen by intestinal bacteria [14]. The available studies do not demonstrate that moderate soybean intake has been associated with an increased risk of infertility, deterioration of semen quality, and a decrease in blood testosterone levels [13, 15]. More studies describing the effect of isoflavones are necessary to work out the effect of soya consumption on fertility.

Therefore, the objective of the work was to evaluate the effect of consumption of soybean on variation in semen parameters related to male fertility in healthy individuals eating a routine diet. The study also aimed to identify potential mechanisms that modulate fertility status. The findings could also be wont to encourage preventative measures against health behaviors that cause reduced fecundity in males. Moreover, knowledge of male reproductive dysfunction will increase awareness to reduce gender inequalities.

MATERIALS AND METHODS

Study design

The effect of soybean enriched diet on semen quality and functionality of males was designed as 60 days, randomized, controlled clinical trial. The data were obtained from people

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visiting the infertility clinic (Kumar Janch Ghar, Sasaram) centre. The study was based on the retrospective review of a total of 2117 (20-59 years old) subjects, who had their semen examined at the clinic between the years 2018 to 2019. All the participants provided written informed consent following instruction of Ethical Committee.

The visit has scheduled a maximum of 15 days after randomization. At the visit, 50g soybeans/individual/day were provided free of charge to needy participants in pre-weighed packs or they were convinced to use soybean as per the given instructions. Detailed dietary instructions including recipes (with or without soybean) were got to the participants to extend adherence to the intervention. At each month, dietary intake was estimated using the mean of 3-d dietary records on a weekend day.

Semen sample analysis

Semen samples were collected by masturbation at baseline and at the end of the intervention period, after 3 d of sexual abstinence.

Analyses of fresh samples have been conducted a maximum of 60 min after collection. The semen was diluted with 10% dimethyl sulfoxide and frozen at −80◦C till any analysis.

The semen parameters, including volume, sperm motility, viability, motility and viscosity were determined on fresh samples [16]. Total sperm count and concentration were determined with a 100 µm–deep hemocytometer chamber (Neubauer chamber), using bright-field microscope (×400 magnification). Sperm motility was assessed under a light microscope (×400 magnifications), Sperm vitality was estimated by evaluating the sperm plasma membrane integrity using eosin-nigrosine (×1000 magnification). Viscosity was observed in by permitting the semen to liquefy using a viscometer. When the liquefaction was complete, the sample was allowed to drop by the force of gravity through a pipette. The length of thread was measured as the semen drops.

Statistical analysis

The data for the participants were shown as means±SDs for normally distributed variables and non-normal continuous variables using GraphPad Prism 5.

Table 1 Parameters of central tendency of semen and sperm parameters by age quartile of males of Sasaram under control diet.

	Age group (years)				Average			
	20-29 (n=290)	**30-39 (n=325)**	**40-49 (n=335)**	**50-59 (n=110)**	**20-59 (n=1060)**	**Range**	**Median**	**Mode**
Semen volume (ml)	4.75	4.57	3.69	3.21	4.06±0.55	2.0 – 8.0	5.00	5.50
Semen viscosity (minute)	34.56	30.13	17.38	17.25	25.20±8.88	13.0 – 40.0	18.0	18.0
Sperm density (million/ml)	34.6	34.5	31.4	30.3	32.7±2.5	30.0 – 45.0	35.0	35.0
Viability (hr)	9.13	8.91	9.82	8.45	8.80±0.54	6.50 – 11.0	8.50	9.0
Motility (%)	50.94	46.77	44.75	44.37	46.71±14.04	10.0 - 75.0	49.0	40.0

Table 2 Parameters of central tendency of semen and sperm parameters by age quartile of males of Sasaram under soybean added diet.

	Age group (years)				Average				ANOVA
	20-29 (n=294)	**30-39 (n=324)**	**40-49 (n=335)**	**50-59 (n=114)**	**20-59 (n=1067)**	**Range**	**Median**	**Mode**	
Semen volume (ml)	3.89	3.11	2.21	2.05	2.89±0.48 [-28.82%]	2.0 – 7.0	3.00	3.00	(C_{df1}=84.69** R_{df3}=40.95**)
Semen viscosity(min)	31.10	27.12	15.64	15.53	22.35±2.75 [-11.31%]	11.0 –37.0	15.0	15.0	(C_{df1}=31.25* R_{df3}=359.5***)
Sperm density (million/ml)	31.1	29.9	26.1	23.2	27.6±4.1 [-15.60%]	25.0 – 30.0	30.0	30.0	(C_{df1}=55.58** R_{df3}=0.31^{NS})
Viability (hr)	8.22	7.67	7.42	6.97	7.57±0.88 [-13.98%]	5.50 –10.0	7.50	7.5	(C_{df1}=53.16** R_{df3}=6.74^{NS})
Motility (%)	74.19	66.68	74.31	63.85	69.88±13.52 [+33.16%]	28.0 –91.0	69.0	60.0	(C_{df1}=100.8** R_{df3}=2.55^{NS})

Table 3 Pair wise correlation coefficients for semen and sperm parameters of males of Sasaram under control diet.

	Semen volume (ml)	**Semen Viscosity (minute)**	**Sperm density (million/ml)**	**Viability of sperms (hr)**	**Motility of sperm (%)**
Semen volume (ml)	1.0	0.959**	0.994***	0.968**	0.415^{NS}
Semen Viscosity (minute)		1.0	0.963**	0.918*	0.267^{NS}
Sperm density (million/ml)			1.0	0.936*	0.320^{NS}
Viability (hr)				1.0	0.614^{NS}
Motility (%)					1.0

(Significance level NS= P>0.05, *= P<0.05, **= P<0.01, ***= P<0.001).

Table 4 Pair wise correlation coefficients for semen and sperm parameters of males of Sasaram under soybean added diet.

	Semen volume (ml)	**Semen Viscosity (minute)**	**Sperm density (million/ml)**	**Viability of sperms (hr)**	**Motility of sperm (%)**
Semen volume (ml)	1.0	0.982**	0.940*	0.956**	0.976**
Semen Viscosity (minute)		1.0	0.945*	0.903*	0.922*
Sperm density (million/ml)			1.0	0.951*	0.864^{NS}
Viability (hr)				1.0	0.946*
Motility (%)					1.0

(Significance level NS= P0.05, *= P<0.05, **= P<0.01, ***= P<0.001).

Two-way ANOVA was applied to assess differences in changes between intervention groups after adjusting for baseline values. Spearman correlation coefficients were wont to calculate pair-wise correlations.

OBSERVATION AND RESULTS

The mean, mode, median, standard deviation and range of seminal parameters and pair-wise correlation for all the 2127 participants of age-group 20-59 years are presented in Tables 1-4. The data showed statistically significant differences in the seminal characteristics of the subjects analyzed between participants feeding on the control diet and soybean added diet in the semen volume, semen viscosity, sperm density, sperm viability and sperm motility.

The observed values of semen volume, semen viscosity, sperm density, sperm viability and sperm motility among the men of Sasaram once feeding control diet were among the above normal ranges. The median represents the location of a set of numerical data using a single number. The median and also mode is not affected by either extreme large or small values. These features were considered throughout the appliance of median and mode in work.

The mean semen volume among men nourishing with control diet was 4.06 ± 0.55ml, which significantly lowered to 2.89 ± 0.48ml after feeding of soybean added nutrition. Similar trend was also observed for semen viscosity (25.20 ± 8.88 versus 22.35 ± 2.75minutes), sperm density (32.7 ± 2.5 versus 27.6 ± 4.1million/ml), sperm viability (8.80 ± 0.54 versus 7.57 ± 0.88hours) but inverse for sperm motility (46.71 ± 14.04 versus 69.88 ± 13.52%) (Tables: 1 and 2).

The decline in semen volume, semen viscosity, sperm density, sperm viability was declined by -28.82%, -11.31%, -15.60%, -13.98% respectively but sperm motility increased by +33.16%. The results of the regression analyses exploring the relationship between semen parameters may be presented as for

(a) Semen volume: $y = 5.980 - 0.055x$ (Routine Diet) ($r = -0.974^{**}$)
$y = 5.242 - 0.067x$ (Soybean added Diet) ($r = -0.962^{**}$)
(b) Semen viscosity: $y = 47.46 – 0.646x$ (Routine Diet) ($r = -0.941^{*}$)
$y = 42.71 – 0.581x$ (Soybean added Diet) ($r = -0.941^{*}$)
(c) Sperm density: $y = 38.3 – 0.016x$ (Routine Diet) ($r = -0.946^{*}$)
$y = 37.2 – 0.027x$ (Soybean added Diet) ($r = -0.982^{**}$)
(d) Sperm viability: $y = 9.583 - 0.022x$ (Routine Diet) ($r = -0.997^{***}$)
$y = 9.270 - 0.040x$ (Soybean added Diet) ($r = -0.991^{***}$)
(e) Sperm motility: $y = 54.31 - 0.217x$ (Routine Diet) ($r = -0.931^{*}$)
$y = 78.45 - 0.244x$ (Soybean added Diet) ($r = -0.589^{NS}$)

The results clearly demonstrated an inverse and linear association of semen volume, semen viscosity, sperm density, sperm viability and sperm motility in control diet versus soybean added diet.

DISCUSSION

India includes a high heterogeneous human population density contributing to approximately 25% of the world population. Infertility is outlined as the failure to achieve a successful pregnancy after 12 months or more of regular unprotected intercourse. Semen is initially thick and viscous; the thicker the semen, the more difficult it is for sperm to travel. Asians consume about 20–80 g soy foods daily in many forms including soybean sprouts, toasted soy protein flours, soy milk, tofu and many more. The amount of soybean given was within the range of given consumption.

The baseline sperm density for Indian men was reported as 68.22±15.14 million/ml [17]. However, generally it ranges from >30million/ml to 81.08±29.21million/ml. The value of baseline sperm density was just more than >30million/ml but less in the soybean treated group. A total motile sperm count over 20 million is taken into account to be normal. In a study, a range from 1.707 to 2.64 ml of semen volume 23.64to 40.26 million/ml of sperm density and 47.14to 61.16% sperm motility among infertile men of South India was investigated [18]. However, there was no significant difference in the motility between the two studies, suggesting a definite decline in the sperm concentration in the southern part of India.

In the present study, we demonstrated that after adding soybean to a routine diet for 60 days, semen volume declined from 4.06 to 2.89 ml, semen viscosity from 25.20 to 22.35 minutes, sperm density from 32.7 to 27.6 million/ml, sperm viability from 8.80 to 7.57 hours but sperm motility increased from 46.71 to 69.88% in a group of participants compared with the control group.

ANOVA inferred that all the changes in semen quality were found significant ($p<0.05$) with both age of the participants and the type of nutrition (Table 2). Pair-wise correlation of semen parameters indicated that semen volume had a positive and significant ($P<0.05$) relationship with semen viscosity, sperm density, sperm viability and sperm motility in participants of both the control diet and soybean added diet (Tables 3 and 4).

Earlier, a decline in sperm volume from 3.15 to 3.00ml, sperm viability from 8.0 to 7.86 hours from the administration of walnuts for 84 days was reported [19]. Conversely, they also reported an increase in sperm density from 21.20 to 26.20 million/ml and a decrease in sperm motility from 67.96 to 64.23%[19]. The reason for the decline in semen quality is presumably due to biological, environmental, nutritional, socioeconomic or other unknown causes [20, 21].

An animal study has demonstrated that high exposure to dietary phytoestrogens present in soybean might end in lower sperm quality. 20 of soy foods is equivalent daily to 25 mg total isoflavone, a most prevailing phytoestrogens and 8 g soy protein [22]. It means that 50g soy food is equivalent to 62.5mg total isoflavone. Fewer sperm concentrations were also observed in men who intake a great many soy foods due to presence of such amount of isoflavone, compared to people who did not consume it [23].

In rodents, exposure to phytoestrogens throughout early post-natal life through diet or subcutaneous injection ends up in multiple reproductive abnormalities during adult life, including slashed testicular weight or size and decreased spermatogenesis [24].

The principal hypothesis for the negative effect of soy foods on male fertility was the phytoestrogen concentration. Phytoestrogens have known deleterious effects on the male endocrine system with potential effects on fertility [25].

CONCLUSION

Our study provides evidence that the quality of human semen is deteriorating in the region of Bihar of India probably due to nutritional causes. This study evaluated the associations between soybean added diet and semen quality among the males of Sasaram. The normal values of semen volume, semen viscosity, sperm density and sperm viability were inversely associated with soybean while sperm motility showed a positive association, while. Findings in this study need to be further investigated in the general population and large sample cohort studies.

Conflict of Interests

The authors have declared that they have no personal, academic, financial, commercial or political competing interest.

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Authors' contributions

Dina Nath Pandit carried out the literature review and drafted the manuscript. Sunil Kumar collected data and helped to draft the manuscript. Anil Kumar Sinha and Mohita Sardana gave the vision and suggestions regarding the importance of the study. Further, all authors contributed to the proofreading, revision and final approval of the manuscript.

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