British Journal of Medicine & Medical Research
19(12): 1-11, 2017; Article no.BJMMR.31150
ISSN: 2231-0614, NLM ID: 101570965

SCIENCEDOMAIN *international*
www.sciencedomain.org

The Effect of Two Types of Statins (Rosuvastatin and Atorvastatin) on the Fertility of Male and Female Mice

Asaad A. Bustan[1*] and Abdullah M. Jawad[1]

[1]Department of Pharmacology, College of Medicine, University of Basrah, Basrah, Iraq.

Authors' contributions

This work was carried out in collaboration between the two authors. Both authors designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. They managed the analyses and the literature searches of the study. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJMMR/2017/31150
Editor(s):
(1) N. Alyautdin Renad, Department of Pharmacology (Pharmaceutical Faculty), I. M. Sechenov MSMU, Moscow, Russia.
Reviewers:
(1) Edy Novery, Padjadjadran University, West Java, Indonesia.
(2) Marie-Christine Raymond Shakib, National Research Centre, Dokki, Cairo, Egypt.
(3) Naufal Zagidullin, Bashkir State Medical University, Russia.
Complete Peer review History: http://www.sciencedomain.org/review-history/17971

Short Research Article

Received 22nd December 2016
Accepted 17th February 2017
Published 27th February 2017

ABSTRACT

Background: Studies showed controversial results regarding the effect of statins on fertility parameters. Several studies present evidence that statins have deleterious effects, others failed to prove these effects.
Aim: To investigate the effects of rosuvastatin and atorvastatin on selected fertility parameters in male and female mice, and to find out their effects on first and second generations of offspring.
Methods: For the first generation studies, mice [30 males and 60 females] were allocated into four groups (6-8 females and 3-4 males, matched for age, in each of the four groups). Rosuvastatin (3.5 mg/kg) or atorvastatin (10.6 mg/kg) was administered once daily for 21 days in a volume of 0.1 ml for each 20 g body weight. Number of live and dead pups, pregnancy success, pups weight, duration of pregnancy, male to female ratio, congenital anomalies, male serum testosterone levels, testes weight and dimensions, and testes histopathology were followed. For the second generation studies, male and female offspring mice were mated and followed for fertility, pregnancy success, and sexual maturity.

**Corresponding author: E-mail: asaadtemimee@gmail.com;*

Results: (i) First generation study: Although the changes in the measured fertility parameters induced by rosuvastatin were not statistically significant, there were trends toward lower number of pups in male and both (male and female) treated groups, lower pups weight in all treated groups, and lower serum testosterone and testes weight and dimensions in male-treated group when compared to control.
Atorvastatin showed a statistically significant reduction in serum testosterone levels (-39.6%), lower testes weight (-13.3%), and mild to moderate histopathological changes in testes of male-treated group in comparison to untreated control group. There was lower number of pups (-6.8%) in male-treated. Pups weight in all treated groups and testes dimensions in male treated group showed trends toward lower values when compared to control.
The male to female (M/F) ratio in the female-treated group was higher in both rosuvastatin experiment (1.17 versus 0.67 for control) and atorvastatin experiment (1.10 versus 0.72 for control).
(ii) Second Generation Study: The only significant finding is longer time to delivery in mice born to treated mothers and fathers. The duration increased by 9.8% and 17% in rosuvastatin and atorvastatin experiments respectively in comparison to untreated control.
Conclusion: Atorvastatin seems to have more harmful effects on fertility than rosuvastatin particularly with respect to their effect on male serum testosterone level and testes weight. The more male to female ratios after treating females with either statin is interesting and worth further investigation.

Keywords: Rosuvastatin; atorvastatin; fertility.

1. INTRODUCTION

Statins might affect testosterone synthesis either by lowering serum LDL cholesterol [1,2] or by inhibiting the *de novo* synthesis of cholesterol [2,3]. A clinical trial on simvastatin (20 and 40 mg) and pravastatin (40mg) administered daily for hypercholesterolemic males, for 24 weeks showed no negative effect on both the amount of testosterone produced by testes and spermatogenesis when compared to placebo [4]. Another clinical trial was performed to study the effect of a higher dose of simvastatin (80 mg) on the level of testosterone found a slight decline in testosterone level without compensatory increase in follicle-stimulating hormone and luteinizing hormone [5]. A review in 2008 stated that several case reports had suggested that statins may be associated with impaired sexual function and that statins might be related to male gynecomastia and oligospermia [6]. A meta-analysis conducted in 2013 showed that there is an evidence that statins lower testosterone in men [7].

The effect of statins on erectile dysfunction was also studied [8-11]. The results are contradictory. Statins were found to improve erectile function. [8-10] or can, in other studies, worsen erectile function [11].

The effects of 4 statins on steroidogenesis, DNA synthesis and viable cell number were studied *in vitro* involving ovarian theca-interstitial cells [12]. It was found that simvastatin and lovastatin decreased all these parameters more than atorvastatin and pravastatin.

The contradictory results and the differences between statins in their effects on fertility led us to conduct this study by selecting two statins with different properties to investigate their effects on the fertility of male, female or both male and female mice.

2. MATERIALS AND METHODS

Mice (a total of 90; 30 males (25-35 g) and 60 females (20-30 g), at age of 12-16 weeks, were kept in plastic cages under laboratory conditions of 25±5Ċ temperature, and fed with standard laboratory pellets *ad libitum* with free access to tap water. Mice were left under these conditions for one week for acclimatization before commencement of experiments. Each animal was tested once only.

2.1 First Generation Studies

For rosuvastatin experiment, 14 male and 28 female mice were used and divided into four groups.

- Control group: 4 untreated male mice and 8 untreated female mice.
- Female fertility study: 3 untreated males and 6 treated females.
- Male fertility study: 3 treated males and 6 untreated females.
- Both male and female fertility study: 4 treated males and 8 treated females.

For atorvastatin experiment, 16 male and 32 female mice were used and divided into 4 groups.

- Control group: 4 untreated male mice and 8 untreated female mice.
- Female fertility study: 4 untreated males and 8 treated females.
- Male fertility study: 4 treated males and 8 untreated females.
- Both male and female fertility study: 4 treated males and 8 treated females.

For the second generation experiment, after weaning, one male and one female offspring were chosen from each mother in the first generation experiment. They were matched for weight and general behavior. At the age of 5 weeks, male and female offspring were used for second generation fertility studies.

For rosuvastatin second generation experiment:

- Control group: 6 males born to untreated male and female mice and 6 females born to untreated male and female mice.
- Female fertility study: 6 males born to untreated males and 6 females born to treated females.
- Male fertility study: 6 males born to treated males and 6 females born to untreated females.
- Both male and female fertility study: 6 males born to treated males and females and 6 females born to treated males and females.

For atorvastatin second generation study:

- Control group: 7 males born to untreated male mice and 7 females born to untreated female mice.
- Female fertility study: 6 males born to untreated males and 6 females born to treated females.
- Male fertility study: 6 males born to treated males and 6 females born to untreated females.
- Both male and female fertility study: 6 males born to treated males and treated females and 6 females born to treated males and treated females.

The extrapolation of doses of the two statins were normalized using an equation advocated by the Food and Drug Administration (FDA) in 2005 [13,14].

Human equivalent dose x Km human=animal dose x Km animal

Where Km (mass constant) is a factor used to convert a dose from mg per kg to mg per square meter.

For a mouse 20 g body weight, the dose of rosuvastatin was 0.07 mg (3.5 mg/kg). The dose was administered in 0.1 ml volume for a 20 g mouse [15]. The dose of atorvastatin was obtained by multiplying the dose of rosuvastatin by 3. Each one male was kept in one cage with 2 females and divided into 4 main groups: Control group, male treated group, female treated group and both male and female treated group.

Drugs (atorvastatin and rosuvastatin) or vehicle were given orally once daily for 2 weeks to males or females separately and for a week after mating then separated.

Serum was taken from treated male animals to measure testosterone level and lipid profile and compared them with control and untreated animals.

Left testes were collected and used for histopathological study. Right testes were collected and weighed and their lengths and widths were measured.

Specimens of the testes from atorvastatin experiment were fixed using Bouin's solution which is known to allow better nuclear staining than 10% formalin. Testes were sectioned using a microtome (Germany) and stained with eosin and hematoxylin. Testes in rosuvastatin experiment were fixed using 10% formalin (instead of Bouin's solution) which had resulted in abnormal changes in both control and rosuvastatin-treated testes. The results of histopathological examination of atorvastatin experiment were categorized as no change, mild, moderate and severe changes.

In the second generation fertility studies, for rosuvastatin experiment, twenty four males and twenty four females at the age of 5 weeks were

mated for 21 days and followed for fertility, sexual maturity and pregnancy success. For atorvastatin experiment, twenty five males and twenty five females at the age of 5 weeks were mated for 21 days and followed for fertility, sexual maturity and pregnancy success.

Analysis of variance (ANOVA) was used to compare between groups using Statistical Package for Social Sciences (SPSS) program version 19. Parametric (Unpaired T-test) and non-parametric (Mann-Whitney, Chi-square) tests were used to test the difference between groups.

The study protocol was reviewed and approved by the postgraduate research committee and the council of Basrah Medical College.

3. RESULTS

3.1 First Generation Studies

3.1.1 The effect of rosuvastatin (3.5 mg per kg for 21 days) on mice fertility

Rosuvastatin showed no statistically significant effect on the measured fertility parameters, serum testosterone level and testes weights and dimensions. No failure of pregnancy was noted in any of the treatment groups.

However, taking the effect size (percent change) in consideration, the following can be identified: treatment of male mice resulted in 10.1% and 15% reduction in the total number of births and pups weights, respectively (Table 1). This is in line with the 18% reduction in serum testosterone level detected in male mice (Table 2). In addition, treatment of females resulted in more male to female ratio (1.17 versus 0.67 for control). Unfortunately the histopathological examination was inconclusive because of technical errors in their preparation.

3.1.1.1 Lipid profile

There was a statistically significant difference between treated and control groups with respect to triglycerides and very low density lipoproteins (VLDL) levels. No statistically significant difference was found in the levels of total cholesterol, LDL and HDL (Table 4).

3.1.2 The effect of atorvastatin (10.6 mg/kg/day for 21 days) on mice fertility

Although there was a statistically significant difference in serum testosterone level (a reduction by 39.6%) (Table 6) and 13.3% reduction in testes weights (Table 7), no failure of pregnancy was noted in any of the treatment groups except one female in the male treated group. No statistically significant effect of atorvastatin on other fertility parameters measured in this study was found. However, taking the effect size (percent change) in consideration, the following can be identified: Treatment of male mice resulted in 6.8% reduction in the total number of births (Table 5). In addition, there was lower testes dimensions in male treated group when compared to control, which, although statistically not significant, is in line with statistically significant low serum testosterone and low testes weights (Tables 6 and 7 respectively). Treatment of females only also resulted in more male to female ratio (1.10 versus 0.72 for control).

Results of histopathological examination are shown in Table 8. Atorvastatin caused mild to moderate abnormal changes in the testicular tissue, and severe in only one mouse. This is compared with mild or no change in control group (Fig. 1A and B).

3.1.2.1 Lipid profile

There was a statistically significant difference between treated and control groups for triglycerides and VLDL levels, while total cholesterol, LDL and HDL did not reach statistical significance (Table 9).

3.1.3 Second generation studies

The potential effect of rosuvastatin on fertility of offspring born to mother and/or father mice treated with rosuvastatin (3.5 mg/kg/day) or atorvastatin (10.6 mg/kg/day) for 21 days: A significant difference was found between offspring born to both mother and father treated mice compared to those born to untreated mice with respect to duration of pregnancy (time from the first day of mating till the day of delivery). The duration increased by 9.8% and 17% in rosuvastatin and atorvastatin experiments respectively in comparison to untreated control.

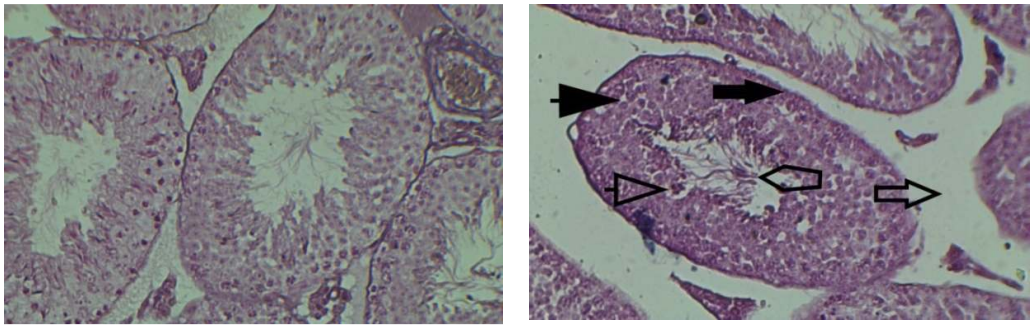
No significant differences were found in other parameters including male/female ratio, and no gross congenital abnormalities were seen in the treated groups.

Table 1. Fertility studies in mice treated with rosuvastatin (3.5 mg/kg/day) for 21 days

Groups	No. of female animals	Total no. of pups/each delivery	Weight of pups (g)	Days to delivery	No. of male pups	No. of female pups
Control (Untreated males mated with untreated females)	6 Untreated	7.00± 1.55	10.82 ± 2.46	22.83±0.98	2.67 ±1.75	4 .00± 2.28
Treated females (mated with untreated males)	6 Treated	6.83± 2.9 (-2.4%)	9.88±3.64 (-8.7%)	22.33± 1.51 (-2.2%)	3.50 ± 2.17 (+31%)	3.00± 1.41 (-25%)
Treated males (mated with untreated females)	7 Untreated	6.29± 1.25 (-10.1%)	9.18 ± 1.66 (-15%)	21.40 ± 0.55 (-6.3%)	2.57 ± 1.62 (-3.7%)	3.71± 1.38 (-7.3%)
Both males and females treated	6 Treated	6.16± 2.99 (-12.8%)	10.32± 3.90 (-4.6%)	23.00± 1.09 (+0.7%)	3.17 ± 2.56 (+18.7%)	3.00± 2.28 (-25%)

*Data are presented as mean ±SD (with percent change with respect to control) Significant difference with respect to control: *P<0.05.*

Note: The sum of the number of males and females pups equals to the number of live pups rather than the total pups since the determination of sex is possible only when the pups are about 25 days of age



(A) **(B)**

Fig. 1. (A) Histolopathology of untreated male mice testes (10X). (B) Histolopathology of testes of male mice treated with atorvastatin (10.6 mg/kg/day) for 21 days (10X)

(Solid arrow: thickening of the tubular walls and an increase in spaces between tubules. Hollow arrow: An increase in space between tubules. Solid triangle: mild changes in appearance of Sertoli cells with presence of intercellular vacuoles. Hollow triangle: There are intercellular vacuoles with presence germinal cells separated from the basement membrane in some tubules. Pentagon: Fibrosis like appearance and aggregation of the germinal cells in tubular lumen

Table 2. Serum testosterone levels in male mice treated with rosuvastatin (3.5 mg/kg/day) for 21 days

Groups	No. of male mice	Serum Testosterone in ng/mL (mean±SEM)	% decrease with respect to control
Untreated males	5	0.39±0.31	-
Treated males	7	0.32±0.16	-18%

Table 3. Weights and dimensions of mice testes treated with rosuvastatin (3.5 mg/kg/day) for 21 days

Groups	No. of male mice	Testes weights (g)	Testes lengths (mm)	Testes widths (mm)
Untreated males	6	0.090±0.014	7.09±0.52	4.54±0.35
Treated males	7	0.085±0.011 (-4.9%)	6.97 ±0.30 (-1.7%)	4.35±0.16 (-4.2%)

Table 4. Lipid profile in male mice treated with rosuvastatin (3.5 mg/kg/day) for 21days

Groups	No. of mice	Total cholesterol	Triglyce-rides	HDL	VLDL	LDL
Untreated males	6	115.3±37.5	144.5±27	40.3±11.7	28. 7±5.4	48±26.6
Treated males	7	119.3±37.4	114.2±17.8	40.1±12	22.9± 3.6	56.3±29
Percent change		+3.5%	-21%*	-0.5%	-20.2%*	+17.2%

*Data are presented as means ±SD (with percent change with respect to control). Significant difference with respect to untreated males: *P<0.05*

Table 5. Fertility studies in mice treated with atorvastatin (10.6 mg/kg/day) for 21 days

Groups	No. of female animals	Total no. of pups/ each delivery	Weight of pups (g)	Days to delivery	No. of male pups	No. of female pups
Control (Untreated males and females were mated)	8 Untreated	6.75±2.38	10.41±2.80	23.38±2.20	3±0.81	4.14±1.95
Treated females (mated with untreated males)	6 Treated	7.00±1.67 (+3.7%)	9.67 ±2.05 (-7.1%)	22.50±0.55 (-3.8%)	3.5±1.5 (+16.7%)	3.17±1.47 (-23.4%)
Treated males (mated with untreated females)	7 Untreated	6.29±2.93 (-6.8%)	9.80 ±1.51 (-5.9%)	23.17±2.32 (-0.9%)	3±0.89 (0%)	4.3±0.81 (- 3.9%)
Both males and females were treated	6 Treated	6.33 ±2.7 (-6.2%)	8.6±3.2 -17.4%	22.17±0.98 (-5.2%)	3.17±1.5 (+5.7%)	2.8±1.3 (-32.4%)

*Data are presented as mean ±SD (with percent change from control) Significant difference with respect to control: *P<0.05. Note: The sum of the number of males and females pups equals to the number of live pups rather than the total pups since the determination of sexes is possible only when the pups are about 25 days of age*

4. DISCUSSION

A number of studies had suggested that statins may have a negative impact on fertility [7,16,17]. Other studies, on the other hand, found no effect for statins on fertility [4,18,19,20].

For these controversial results and because statins are commonly prescribed drugs, their effects on fertility of male, female, and both male and female mice had been investigated in this study, using two statins with different properties; atorvastatin and rosuvastatin.

These effects on fertility may not be related to cholesterol (pleiotropic effects), since statins are shown to have no cholesterol lowering effect in rodents [21]. The results of the present study also showed that rosuvastatin and atorvastatin did not affect cholesterol level but reduced triglycerides and VLDL levels. However, the presence of cholesterol lowering effect in human may further complicate the final effect on fertility.

Table 6. Serum testosterone levels in male mice treated with atorvastatin (10.6 /kg/day) for 21 days

Groups	No. of male mice	Serum testosterone in ng/ml (mean ±SEM)	% decrease with respect to control
Untreated males	6	0.48±0.05	
Treated males	7	0.29±0.04	39.6%* -

*Significant difference with respect to control: *P<0.05*

Table 7. Weights and dimensions of male mice testes treated with atorvastatin (10.6 /kg/day) for 21 days

Groups	No. of male mice	Testes weights (g)	Testes lengths (mm)	Testes widths (mm)
Untreated males	7	0.09 ±0.01	7.07±0.74	4.55±0.32
Treated males	7	0.08 ± 0.01 (-13.3%)*	6.60±0.36 (-6.65%)	4.33±0.23 (-4.8%)

*Data are presented as means ±SD (with percent change with respect to control). Significant difference with respect to control: *P<0.05*

Table 8. Scores of histopathological changes in testes of male mice treated with atorvastatin* (10.6 mg/kg/day) for 21 days

Group (No. of mice)	No. of sections examined	Scores of histopathological changes			
		0 (No apparent changes)	1 (mild changes)	2 (Moderate changes)	3 (Severe changes)
Control (n=7)	7	4	3	0	0
Atorvastatin treated (n=7)	**7**	**0**	**3**	**3**	**1**

** A technical error (using formalin as a fixative agent) resulted in damage of the specimens of rosuvastatin experiment*

Table 9. Lipid profile in male mice treated with atorvastatin (10.6 mg/kg/day) for 21 days

Groups	No. of male mice	Total cholesterol	Tri-glycerides	HDL	VLDL	LDL
Untreated males	7	146.9±55.2	139.7±26	39.3±22	27.7±5.1	79.9±32.6
Treated males	7	120.7±31	107±19.4	29.6±11	21.1±3.7	68.6±20.6
Percent change		-17.8%	-23.4%*	-24.7%	-23.8%*	-14.1%

*Data are presented as means ±SD (with percent change with respect to control). Significant difference with respect to control: *P<0.05*

Taking into account previous reports of testicular degeneration caused by statins such as simvastatin [22] and lovastatin [23] and the suggestion of several later studies that statins had an effect on testes weights, may lead to the conclusion that these changes in the testes might be related to the drug itself. Of these studies is a study conducted in Parke-Davis laboratories, in 2012 and cited by Klinefelter et al. [16] which reported that high-dose atorvastatin resulted in low testes weights in rats among other abnormalities such as low sperms count, low sperm motility and an increase in abnormal sperms in semen. Another study reported dose dependent reduction in testes volume together with symptoms of hypogonadism in 244 patients with erectile dysfunction treated with statins; the majority of them were on atorvastatin [24]. The findings of these studies are in agreement with results of the present study, where significantly lower testes weights were found in male mice treated with atorvastatin.

In contrast, an increase in the testes weights of hypercholesterolemic rats was reported [25]. The differences in the direction of the effect on testes weights (a decrease or an increase) may be speculated to be related to species variation, health status or to properties of statins.

Kilnefelter et al. in 2014, studied the *in vitro* effect of 3 statins, atorvastatin, simvastatin and mevastatin on rat Leydig cells. They found that these drugs inhibited the production of testosterone at a low concentration (0.3 µM) [16].

Atorvastatin, used in a dose of 20mg/day or more, in diabetic type-2 patients resulted in low total but not in the bioavailable (free plus albumin bound) testosterone [26]. However, there are studies presenting evidence that testosterone bound to sex hormones binding globulin (SHBG) is also biologically active [27,28].

Clinical studies conducted by Kanat et al. in 2009 and Krysiak et al. in 2014 and 2015, reported deleterious effect of statins (atorvastatin and rosuvastatin) on gonadal hormones [29-31]. However these studies suggested different conclusions regarding the correlation of this effect with cholesterol level, where Kanat el al. suggested that the hormonal effect is related to the cholesterol lowering effect of statins [29], while Krysiak et al. [31] presented evidence correlating the hormonal effect to the pleiotropic effect of these drugs. The present study may be in agreement with the conclusion of Krysiak et al. [31] where no cholesterol lowering effect was found in this study.

Smals et al. [20] conducted an *in vitro* study to investigate the effect of simvastatin on homogenates of human testes. They concluded that simvastatin decreased androgen synthesis at a concentration of 1 µM or more, which is equivalent to 4-5 times the dose used in clinical practice. However, equivalent effect might be seen with lower doses using the more potent statins, atorvastatin and rosuvastatin, where atorvastatin is 2.5-4 times, and rosuvastatin is 4-8 times more potent than simvastatin [32,33]. Smals et al. [20] introduced an evidence that the mechanism of inhibiting androgen synthesis by statins was a non-cholesterol lowering mechanism, through inhibition of the enzyme 17-ketosteroid-oxidoreductase. The present study also found that the effects on fertility parameters were not associated with reduction in cholesterol level.

Interestingly, a study conducted by Pons-Rejraji and his co-workers [17], concluded that atorvastatin at a low dose (10mg/day) impaired sperm parameters in normocholersterolemic young subjects without influencing serum testosterone and gonadotropins. The effect of this low dose atorvastatin could be more pronounced in elderly and/or patients with hypogonadism or other health issues. Pons-Rejraji and his co-workers in 2014 [17] indicated that the effect of this low dose of atorvastatin could be related to reduction of intra-testicular androgen synthesis and this reduction is minimal so that it did not affect systemic hormone levels.

Rosuvastatin, in a daily dose of 3-10 mg/kg of juvenile rats body weights, which is calculated to be equivalent to human child daily dose, caused delay in juvenile rat puberty (indicated by a delay in preputial separation, a process which is androgen dependent), a delay in the onset of spermatogenesis, a decrease in sperm formation per day, a decrease in androgen receptors, and epididymal and testicular structural changes, as well as non-significant decrease in serum testosterone [34] This might imply that the trends toward lower number of pups and lower serum testosterone level found in the present study are a true and not a chance effect.

Leite et al. [34] found histopathological changes in seminiferous tubules and germinal cells death associated with the use of rosuvastatin in rats in doses that are comparable or higher than the doses used in the present study. Because of technical error, the findings of Leite et al. [34] cannot be ascertained. On the other hand, Das and Indira [35], found atorvastatin to cause histopathological changes (edema of the interstitium) in rats testes. They related these changes to the androgen lowering effect of atorvastatin, a finding which is in agreement with the results of the present study.

Sokalska et al. [12] presented evidence that statins can affect female fertility. They reported more pronounced effect of lovastatin and simvastatin on steroidogenesis, DNA synthesis and viable cell number than atorvastatin *in vitro* study involving ovarian theca-interstitial cells. In the present study, higher male/female ratio was noticed when females were treated with either rosuvastatin or atorvastatin. This high male/female ratio is interesting and can stimulate more investigation, since there are reports that link hormonal levels to changes in male/female ratio. Different levels of estrogen, testosterone, gonadotropins and progesterone were associated with changes in this ratio. These variations in sex ratio were linked to variations in hormones levels caused or associated with chemicals (e.g. dioxin, fungicides), disease states (e.g. testicular cancer), diet (e.g. fats), infections (parasitic and viral infections), gestational age, time of conception [36], and female position of uterus [37].

In second generation studies, both rosuvastatin and atorvastatin produced a significant effect on the time to conception and/or the duration of pregnancy in mice born to treated mothers and fathers. Atorvastatin effect was more prominent and the delay in time to delivery was longer than

in rosuvastatin experiment. Although the delay was only for few days, it is relatively long enough compared with rodent life span and when extrapolated to human beings. Other fertility parameters were not affected by these two statins.

The fact that the effect appeared only in mice born to groups in which both mothers and fathers were treated, might refer to a mild effect, since such effect did not appear in male-treated groups or in female-treated groups alone. This effect might be interpreted as a delayed maturity. Although this delay is difficult to explain when male fertility is considered, however in female mice, the estrous cycle is only 4-5 days and the pregnancy duration is only 19-21 days [38]. This may indicate that the 4.3-day delay (in case of atorvastatin) and even the 2-day delay (in case of rosuvastatin) are long enough to be taken in consideration as an effect on offspring fertility.

The proposed trans-generational effect of statins can be attributed to one of the established mechanism of action of these drugs which is the epigenetic mechanism [39,40]. This involves statins action on histone deacetylase which functions as gene modulator and regulator [41].

5. CONCLUSION

The findings of the present study may strengthen the suggestion that statins do have an influence on fertility, and that different statins may differ from each other in this respect probably due to differences in their pharmacokinetic properties. Furthermore, the trans-generational effect, may suggest other mechanisms through which statins can affect fertility.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Gwynne JT, Strauss JF. The role of lipoproteins in steroidogenesis and cholesterol metabolism in steroidogenic glands. Endocrine Reviews. 1982;3(3): 299-32.
2. Farnsworth W, Hoeg J, Maher M, Brittain E, Sherins R, Brewer Jr H. Testicular function in type II hyperlipoproteinemic patients treated with lovastatin (mevinolin) or neomycin. The Journal of Clinical Endocrinology & Metabolism. 1987;65(3): 546-50.
3. Alberts A, Chen J, Kuron G, Hunt V, Huff J, Hoffman C, et al. Mevinolin: A highly potent competitive inhibitor of hydroxymethylglutaryl-coenzyme A reductase and a cholesterol-lowering agent. Proceedings of the National Academy of Sciences. 1980;77(7):3957-61.
4. Dobs AS, Miller S, Neri G, Weiss S, Tate AC, Shapiro DR, et al. Effects of simvastatin and prevastatin on gonadal function in male hypercholesterolemic patients. Metabolism. 2000;49(1):115-21.
5. Dobs AS, Schrott H, Davidson MH, Bays H, Stein EA, Kush D, et al. Effects of high-dose simvastatin on adrenal and gonadal steroidogenesis in men with hypercholesterolemia. Metabolism. 2000; 49(9):1234-8.
6. Golomb BA, Evans MA. Statin adverse effects. American Journal of Cardiovascular Drugs. 2008;8(6):373-418.
7. Schooling CM, Yeung SLA, Freeman G, Cowling BJ. The effect of statins on testosterone in men and women, a systematic review and meta-analysis of randomized controlled trials. BMC Medicine. 2013;11(1):1.
8. Doğru MT, Başar MM, Şimşek A, Yuvanç E, Güneri M, Ebinç H, et al. Effects of statin treatment on serum sex steroids levels and autonomic and erectile function. Urology. 2008;71(4):703-7.
9. Cui Y, Zong H, Yan H, Zhang Y. The effect of statins on erectile dysfunction: A systematic review and meta-analysis. The Journal of Sexual Medicine. 2014;11(6): 1367-75.
10. Kostis JB, Dobrzynski JM. The effect of statins on erectile dysfunction: A meta-analysis of randomized trials. The journal of sexual medicine. 2014;11(7): 1626-35.

11. Figueiredo L, Coutinho P, Neves E, Tomada I, Tomada N. MP-07.17 The impact of statins in erectile dysfunction. Urology. 2011;78(3):S81-S2.
12. Sokalska A, Stanley SD, Villanueva JA, Ortega I, Duleba AJ. Comparison of effects of different statins on growth and steroidogenesis of rat ovarian theca-interstitial cells. Biology of Reproduction. 2014;90(2):44.
13. Reagan-Shaw S, Nihal M, Ahmad N. Dose translation from animal to human studies revisited. The FASEB Journal. 2008;22(3): 659-61.
14. Kumari M, Singh P. Study on the reproductive organs and fertility of the male mice following administration of metronidazole. Cell J (Yakhteh). 2013;7(3).
15. Oghenesuvwe EE, Nwoke EE, Latonna AD. Guidelines on dosage calculation and stock solution preparation in experimental animals' studies. Journal of Natural Sciences Research. 2014;18 (4):100-106.
16. Klinefelter G, Laskey J, Amann R. Statin drugs markedly inhibit testosterone production by rat Leydig cells *in vitro*: Implications for men. Reproductive Toxicology. 2014;45:52-8.
17. Pons-Rejraji H, Brugnon F, Sion B, Maqdasy S, Gouby G, Pereira B, et al. Evaluation of atorvastatin efficacy and toxicity on spermatozoa, accessory glands and gonadal hormones of healthy men: A pilot prospective clinical trial. Reproductive Biology and Endocrinology. 2014;12(1):1.
18. Tobert J, Bell G, Birtwell J, James I, Kukovetz W, Pryor J, et al. Cholesterol-lowering effect of mevinolin, an inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme a reductase, in healthy volunteers. Journal of Clinical Investigation. 1982;69(4):913.
19. 19.Dobs AS, Sarma PS, Schteingart D. Long-term endocrine function in hypercholesterolemic patients treated with pravastatin, a new 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor. Metabolism. 1993;42(9):1146-52.
20. Smals AG, Weusten JJ, Benraad TJ. The HMG-CoA reductase inhibitor simvastatin suppresses human testicular testosterone synthesis in vitro by a selective inhibitory effect on 17-ketosteroid-oxidoreductase enzyme activity. The Journal of Steroid Biochemistry and Molecular Biology. 1991; 38(4):465-8.
21. Pecoraro V, Moja L, Dall'Olmo L, Cappellini G, Garattini S. Most appropriate animal models to study the efficacy of statins: A systematic review. European Journal of Clinical Investigation. 2014; 44(9):848-71.
22. Gerson RJ, Macdonald JS, Alberts AW, Kornbrust DJ, Majka JA, Stubbs RJ, et al. Animal safety and toxicology of simvastatin and related hydroxy-methylglutaryl-coenzyme A reductase inhibitors. The American Journal of Medicine. 1989;87: S28-S38.
23. MacDonald JS, Gerson RJ, Kornbrust DJ, Kloss MW, Prahalada S, Berry PH, et al. Preclinical evaluation of lovastatin. The American Journal of Cardiology. 1988; 62(15):J16-J27.
24. Corona G, Boddi V, Balercia G, Rastrelli G, De Vita G, Sforza A, et al. The effect of statin therapy on testosterone levels in subjects consulting for erectile dysfunction. The Journal of Sexual Medicine. 2010; 7(4pt1):1547-56.
25. Shalaby M, El Zorba H, Kamel GM. Effect of α-tocopherol and simvastatin on male fertility in hypercholesterolemic rats. Pharmacological Research. 2004;50(2): 137-42.
26. Stanworth RD, Kapoor D, Channer KS, Jones TH. Statin therapy is associated with lower total but not bioavailable or free testosterone in men with type 2 diabetes. Diabetes Care. 2009;32(4):541-6.
27. Hammes A, Andreassen TK, Spoelgen R, Raila J, Hubner N, Schulz H, et al. Role of endocytosis in cellular uptake of sex steroids. Cell. 2005;122(5):751-62.
28. Rosner W, Hryb DJ, Kahn SM, Nakhla AM, Romas NA. Interactions of sex hormone-binding globulin with target cells. Molecular and Cellular Endocrinology. 2010;316(1): 79-85.
29. Kanat M, Serin E, Tunckale A, Yildiz O, Sahin S, Bolayırlı M, et al. A multi-center, open label, crossover designed prospective study evaluating the effects of lipid lowering treatment on steroid synthesis in patients with Type 2 diabetes (MODEST Study). Journal of Endocrinological Investigation. 2009; 32(10):852-6.
30. Krysiak R, Okopien B. The effect of aggressive rosuvastatin treatment on steroid hormone production in men with coronary artery disease. Basic & Clinical Pharmacology & Toxicology. 2014;114(4): 330-5.

31. Krysiak R, Kowalska B, Żmuda W, Okopień B. The effect of ezetimibe-statin combination on steroid hormone production in men with coronary artery disease and low cholesterol levels. Pharmacological Reports. 2015;67(2):305-9.
32. Jones PH, Davidson MH, Stein EA, Bays HE, McKenney JM, Miller E, et al. Comparison of the efficacy and safety of rosuvastatin versus atorvastatin, simvastatin, and pravastatin across doses (STELLAR Trial). The American Journal of Cardiology. 2003;92(2):152-60.
33. Blasetto JW, Stein EA, Brown WV, Chitra R, Raza A. Efficacy of rosuvastatin compared with other statins at selected starting doses in hypercholesterolemic patients and in special population groups. The American Journal of Cardiology. 2003;91(5):3-10.
34. Leite GAA, de Lima Rosa J, Sanabria M, Cavariani MM, Franci JAA, Pinheiro PFF, et al. Delayed reproductive development in pubertal male rats exposed to the hypolipemiant agent rosuvastatin since prepuberty. Reproductive Toxicology. 2014;44:93-103.
35. Das SS, Indira M. Combined effects of atorvastatin and nicotine on testicular expression of steroidogenic enzymes in experimental rats. International Journal of Pharmaceutical Sciences and Research. 2015;6(6):2511.
36. Michelmann H, Gratz G, Hinney B. XY sperm selection: fact or fiction? Human Reproduction & Genetic Ethics; 2015.
37. James WH. Further evidence that mammalian sex ratios at birth are partially controlled by parental hormone levels around the time of conception. Human Reproduction. 2004;19(6):1250-6.
38. Caligioni CS. Assessing reproductive status/stages in mice. Current Protocols in Neuroscience. 2009;A. 4I. 1-A. 4I. 8.
39. Csoka AB, Szyf M. Epigenetic side-effects of common pharmaceuticals: A potential new field in medicine and pharmacology. Medical Hypotheses. 2009;73(5):770-80.
40. Kato N, Liang Y-Q, Ochiai Y, Jesmin S. Systemic evaluation of gene expression changes in major target organs induced by atorvastatin. European Journal of Pharmacology. 2008;584(2): 376-89.
41. Ordovás JM, Smith CE. Epigenetics and cardiovascular disease. Nature Reviews Cardiology. 2010;7(9):510-9.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/17971