Reprint from The Review of DIABETIC STUDIES Vol 13 No 1 2016

The Review of DIABETIC STUDIES

ORIGINAL DATA

Metformin Treatment Does Not Affect Testicular Size in Offspring Born to Mothers with Gestational Diabetes

Kristiina Tertti[1], Jorma Toppari[2], Helena E. Virtanen[2], Sergey Sadov[2], and Tapani Rönnemaa[3]

[1] *Department of Obstetrics and Gynecology, University of Turku and Turku University Hospital, Turku, Finland.* [2] *Departments of Physiology and Pediatrics, University of Turku, Turku, Finland.* [3] *Department of Medicine, University of Turku and Turku University Hospital, Turku, Finland. Address correspondence to: Kristiina Tertti, Turku University Hospital, Kiinamyllynkatu 4-8, 20520 Turku, Finland, e-mail: kristiina.tertti@tyks.fi*

Manuscript submitted July 12, 2015; resubmitted July 29, 2015; accepted August 4, 2015

■ Abstract

OBJECTIVES: Studies in rodents suggest that metformin treatment during pregnancy may have harmful effects on testicular development in offspring. Our aim was to determine whether metformin treatment of gestational diabetes mellitus (GDM) affects testicular size in male offspring. **METHODS**: We compared the testicular size in prepubertal boys born to mothers who participated in a randomized controlled trial (RCT) comparing metformin with insulin in the treatment of GDM. Twenty-five (42.4% of invited) and 27 (52.9% of invited) boys whose mothers had been treated with metformin or insulin, respectively, participated in the study. Testicular size was measured by a ruler, an orchidometer, and by ultrasonography at the age of 33 to 85 months. **RESULTS**: The mean age of the boys was 60 months at the time of examination, and did not differ between the metformin and insulin group ($p = 0.88$). There was no difference in testicular size between the boys in the two groups (p always ≥ 0.40), and there were no significant differences in height, weight, BMI, BMI z-score, or waist-to-hip ratio (WHR) between the boys in the groups. **CONCLUSIONS**: Prepubertal testicular size did not differ between offspring born to metformin-treated mothers and those born to insulin-treated mothers.

Keywords: gestational diabetes mellitus · metformin · insulin · offspring · testis · testicular size

1. Introduction

Metformin is increasingly being used as an alternative medication to insulin in patients with gestational diabetes mellitus (GDM). To date, metformin has not been associated with a higher risk for perinatal or neonatal adverse outcomes in randomized studies comparing metformin with insulin in GDM patients [1-7]. Metformin crosses the placenta resulting in similar concentrations in fetal and maternal circulation [8, 9]. Knowledge on long-term effects in children exposed to metformin *in utero* is limited.

In polycystic ovary syndrome (PCOS), there are studies in infants of mothers who had been treated with metformin during pregnancy [10-12]. Compared with placebo, one of these studies reported no adverse effects of metformin on growth or motor-social development by the age of 18 months [10]. In the study by Rø *et al.* (2012), height, weight, fat composition, and insulin sensitivity were similar at the age of seven to nine years [11]. Another study reported that the metformin-exposed children weighed more than controls at the age of one year [12].

In a follow-up study in children of GDM patients treated with metformin, a more favorable fat distribution was observed at the age of two years [13]. Another study reported that the children of the metformin-treated patients showed in-

 DOI 10.1900/RDS.2016.13.59

creased weight and height at the ages of 12 and 18 months compared with the children of the insulin-treated patients [14].

Animal studies have raised a concern that metformin may have harmful effects on developing testes [15]. In the study by Tartarin *et al*. (2012), metformin caused a decrease in testosterone secretion compared with controls in organotypic cultures of human and murine testicular tissue [15]. Furthermore, administration of metformin to pregnant mice reduced the size of fetal and neonatal testes compared with controls [15]. There are no reports on the effects of antenatal metformin exposure on the development of human testes. Therefore, we performed a follow-up study in male offspring of GDM patients to determine whether the size of testes is affected by metformin treatment compared with insulin treatment during pregnancy. The boys were recruited from the children of 217 GDM patients who had recently participated in a randomized controlled trial (RCT) comparing metformin with insulin in the treatment of GDM [2].

2. Methods and subjects

2.1 Study design and patients

This is a follow-up study in the male offspring from the previously published study [2]. Briefly, an open-label, randomized, clinical trial comparing metformin with insulin in the treatment of GDM patients was conducted between June 2006 and December 2010 at Turku University Hospital, Turku, Finland. The trial comprised 217 patients at 22-34 weeks of gestation. A hundred and ten patients were allocated to metformin and 107 to insulin treatment (**Figure 1**). In the metformin group, 23 (20.9%) patients needed additional insulin to maintain adequate glycemic control. The median daily metformin dose was 1500 mg.

The study was approved by the Ethics Committee of the Hospital District of Southwest Finland, the Finnish Medicines Agency, and the European Union Drug Regulatory Agency (EUDRA). The trial was registered at Clinicaltrials.gov, NCT01240 785, http://clinicaltrials.gov/ct2/show/NCT01240785. All patients gave their informed consent prior to their participation in the study. The present follow-up study was also approved by the Ethics Committee of the Hospital District of Southwest Finland, and the caretakers gave their informed consent for their children's participation in the study.

Abbreviations:

BMI	body mass index (kg/m^2)
BMI z-score	BMI standard deviation (SD) score (measure of relative weight adjusted for child age and sex)
GDM	gestational diabetes mellitus
gw	gestational week
HbA1c	glycated hemoglobin (hemoglobin A1c)
PCOS	polycystic ovary syndrome
RCT	randomized controlled trial
SD	standard deviation
US	ultrasonography
WHR	waist-to-hip ratio

In the present follow-up study, an invitation letter was sent to all 110 GDM mothers who delivered a male infant, and who were treated with metformin (n = 59) or insulin (n = 51) (**Figure 1**). Fifty-two (47.3% of those invited; 42.4% of those in the metformin group, and 52.9 % of those in the insulin group) boys participated in the present study. Of the 52 boys attending the study, 25 were exposed to metformin *in utero*, and 27 were born to mothers treated with insulin during pregnancy.

The boys in the initial RCT [2] who were not involved in the present study (n = 58), did not differ from the boys who participated (n = 52) with respect to maternal (age, primiparity, smoking, BMI, fasting glucose, HbA1c) or neonatal baseline data (birth weight, prematurity, umbilical artery pH, neonatal hypoglycemia), with p-values always > 0.27. There was a slightly significant difference in gestational weeks (gw) at delivery: mean gw of participants was 39.44 and of non-participants 39.09 (p = 0.045), corresponding to a difference of approximately two days.

2.2 Clinical examinations

Clinical examinations included the measurement of height, weight, and waist and hip circumference. Furthermore, testicular size and urogenital status was recorded, including testicular descent as GDM has been associated with an increased risk for abnormal testicular descent, i.e. cryptorchidism [16]. Twenty-five boys were exposed to metformin *in utero*, and 27 boys were born to mothers treated with insulin during pregnancy.

Body mass index (BMI) was calculated as kg/m^2 and expressed as BMI z-score [17], i.e. a standard deviation score that indicates how many SD-units a child's BMI is above or below the average BMI value according to age and sex. Waist-to-hip ratio (WHR) was calculated as part of the anthropometric measurements.

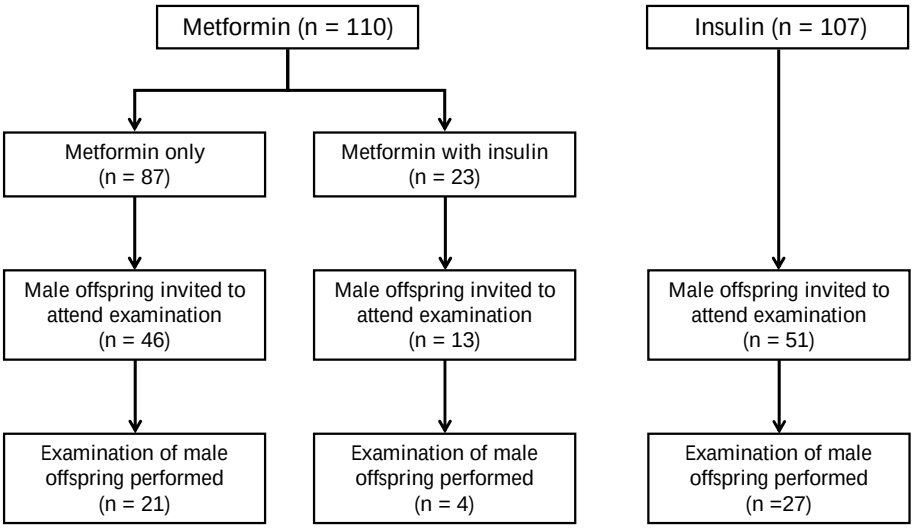

Figure 1. Flow chart of study design.

Maternal smoking during pregnancy was recorded as it has been reported to be associated with congenital cryptorchidism [18] and diminished testicular size [19].

The clinical examination of the boys was performed by trained andrologists (SS or HV) at the Andrology Unit of the Department of Physiology, University of Turku, between July 2013 and March 2014. During the examination, SS and HV had no information about the maternal GDM treatment group. The height of the boys was measured three times using the Harpenden Stadiometer with VR High Speed Counter (Holtain LTD, Great Britain), and weight was measured using the Detecto EU522 Digital Scale (Cardinal Scale Manufacturing Co, USA). In these measurements, the mean of three values was used in the calculation.

Waist and hip circumference was measured once in a standing position with a non-elastic tape. Waist circumference was measured at the end of exhalation at the horizontal level of the midpoint between the lowest rib and iliac crest [20]. Hip circumference was measured at the horizontal level of the widest part of the buttocks. The boys wore only underpants during these measurements.

In the urogenital examination, the position of the testis was assessed to detect possible cryptorchidism and retractile testis. The testicular size was measured by ultrasonography (US) examination (using Aloka Prosound6, Aloka Co., Ltd, Japan, with 13 MHz linear transducer), with plastic ruler (length and width of the testis were measured), and with a Prader's orchidometer at the precision of 0.25 ml (with 12 wooden beads ranging from 1 ml to 25 ml, intermediate values estimated).

In the ultrasonography examination, length, height, and width of the testis were measured three times, and mean length, width, and height were used in the calculations. Testicular volume was calculated by Lambert's formula (0.71 x length x width x height) based on the ultrasonography examination, and by ellipsoid formula (0.52 x length x width^2) based on the ruler measurements. The parents were interviewed about the current medication and diseases of the boys; their answers were recorded.

2.3 Statistical analysis

Statistical analyses were performed by using GraphPad Prism version 5.00 for Windows (GraphPad Software Inc., La Jolla, CA, USA). The normality of distributions was tested with the Shapiro-Wilk normality test. Continuous variables were compared between the groups using the Mann-Whitney U-test or unpaired t-test, as appropriate. For the categorical variable (maternal smoking), Fisher's exact test was used. The two-tail level of statistical significance was set at $p < 0.05$.

A secondary power-calculation was carried out to determine the difference in testicular size (between boys born to mothers in the metformin and those born to mother in the insulin group) which can be excluded with 80% power and a significance level of 0.05 when the group size was taken as 26 per group. In the calculation, we used ultrasound volume data of the left testis, mean 0.90 ml and SD 0.30 ml.

3. Results

The results are shown in **Table 1**. The size of testes evaluated by a ruler, an orchidometer, and by ultrasonography was similar in the metformin

and the insulin group (p always ≥ 0.40). A secondary power calculation revealed that the sample size was sufficient to exclude a 0.24 ml difference in testicular size determined by ultrasonography between boys in the metformin and insulin group. Mean age of the boys was 60 months, age varied from 33 to 85 months at the time of examination, and it did not differ between the groups (p = 0.88). There were no differences in height, weight, BMI, BMI z-score, and WHR of the boys between the groups (p = 0.95, p = 0.61, p = 0.11, p = 0.16, p = 0.32, respectively). There was no significant difference in the prevalence of maternal smoking during gestation between the metformin (n = 4/25, 16.0%) and insulin (n = 3/26, 11.5%) group (p= 0.70, RR 1.2, 95% CI 0.59-2.44).

One boy had been operated on for congenital bilateral cryptorchidism at the age of one year. His 22-year-old mother was treated with metformin during pregnancy, and she smoked. At the time of examination, the right testis was not detected in the scrotum, and the boy was referred for surgery. Furthermore, one boy was diagnosed with rectractile testis (testis saltans) during the examination, and another boy had previously been operated on for retractile testis at the age of four years. The former of the boys belonged to the insulin group and the latter to the metformin group.

Table 1. Clinical data of male offspring aged 33 to 85 months

	Metformin (n = 25)	Insulin (n = 27)	p
Age (mo)	60.4 ± 17.0	60.7 ± 15.4	0.88
Height (cm)	112.5 ± 10.1	112.3 ± 10.4	0.95
Weight (kg)	21.2 ± 5.2	20.2 ± 4.9	0.61
BMI (kg/m^2)	16.4 ± 2.1	15.5 ± 1.5	0.11
BMI z-score[1]	0.25 ± 1.5	-0.22 ± 1.3	0.16
WHR	0.92 ± 0.05	0.91 ± 0.08	0.32
Testis volume (ml)[2]			
Right	1.84 ± 0.6[5]	1.80 ± 0.4	0.86
Left	1.81 ± 0.4	1.78 ± 0.3	0.74
Testis volume by US (ml)[3]			
Right	0.89 ± 0.4[5]	0.87 ± 0.3	0.92
Left	0.93 ± 0.3	0.86 ± 0.3	0.40
Testis volume by ruler (ml)[4]			
Right	1.74 ± 0.74[5]	1.67 ± 0.45	0.90
Left	1.72 ± 0.53	1.60 ± 0.43	0.50

Legend: Data are expressed as mean ± SD, or n (%). [1] BMI standard deviation (SD) score (measure of relative weight adjusted for child age and sex). [2] Measured by Prader's orchidometer. [3] Calculated by using Lambert's formula (0.71 x length x width x height). [4] Calculated by using the ellipsoid formula (0.52 x length x $width^2$). [5] n = 24 (one boy had an undetectable right testis due to cryptorchidism). *Abbreviations*: BMI – body mass index, WHR – waist-to-hip ratio, US – ultrasonography.

4. Discussion

In the present study, we found no difference in the testicular size of prepubertal offspring of mothers treated for GDM with either metformin or insulin. The age of the boys varied from 33 to 85 months at the time of examination; mean age did not differ between the two groups. The oldest boy examined in our study was aged seven. In a cross-sectional study, the size of the testis did not increase significantly during this time window, and did not correlate with height and weight [21].

Testicular measurements were performed by three methods: ruler, Prader's orchidometer, and ultrasonography. These methods are widely used and accepted to evaluate children's testicular size [22]. The size of the testes in our study is comparable with measurements in other cross-sectional studies in the normal population [22, 23], and with our results on testicular size measured with an orchidometer in eight year old prepubertal Finnish boys whose mothers did not have gestational diabetes (unpublished data).

In the study by Tartarin *et al.* (2012), metformin decreased testosterone secretion in organotypic cultures of human fetal testicular tissue compared to control cultures [15]. In cultures of mouse fetal testes, testosterone secretion was decreased only when dosages higher than the therapeutic concentration were used. Furthermore, administration of metformin to pregnant mice during early pregnancy reduced the size of the fetal and neonatal testes compared to controls, but testicular testosterone concentration of newborn mice was not reduced. The number of germ cells was not affected, but the number of Sertoli cells was reduced [15]. Metformin also caused a dose-dependent decrease in cell proliferation in cultures of neonatal mouse Sertoli cells [15]. Sertoli cells are located in seminiferous tubules; they support the development of germ cells. Cell cultures from male infant testes are not available to study the effects of metformin. However, evaluating the size of the testes is considered as a relevant proxy, since there is a strong correlation between the number of Sertoli cells and testicular volume [15].

In a study of mice, it has been observed that offspring of metformin-treated mice fed a normal diet during pregnancy were lighter at birth, but when the male offspring were fed a high-fat diet postnatally, they gained more fat and developed glucose

intolerance compared to controls [24]. In contrast, the offspring of mice that were fed a high-fat diet prior to and during gestation and treated with metformin, gained less body weight and adipose tissue than control mice during a period of a high-fat diet at adult age [25]. In a follow-up of the MiG-study, it was shown that at the age of two years children born to GDM mothers and exposed to metformin *in utero* had larger amounts of subcutaneous fat, but overall body fat was the same as in children whose mothers were treated with insulin alone [13]. In our study, there were no differences in height, weight, BMI, or WHR measurements of the boys between the metformin and insulin group.

Despite the absence of effects on testis size, it cannot be excluded that the hormonal function of the testis has been influenced by metformin. This is a limitation of this study. The options for measuring testicular function at prepubertal age, irrespective of the so-called mini-puberty, are limited. Mini-puberty occurs during the first months of life when the hypothalamic-pituitary-testicular axis is very active and testicular hormone secretion soars [26, 27]. The testosterone level declines to a very low level before the age of six months [26], and starts to increase again at the time of puberty. In our study, the age of examined boys (33 to 85 months) was outside this time frame.

Preliminary human evidence has shown that male and female steroid levels in metformin-exposed offspring do not differ from those in non-exposed controls [28]. In the study by Tartarin *et al*., testosterone secretion in organ cultures of testes from fetuses aborted in the first trimester was suppressed by metformin [15]. The first trimester of pregnancy is the time at which the gonads are most vulnerable to exogenous substances, as the first step of gonadal differentiation occurs at this time. However, our patients were exposed to metformin from gestational week 22 onwards only [2], i.e. after the most critical time. The medication for GDM patients is usually started after the first trimester. However, fetal exposure to metformin from the first trimester onwards is possible, namely in the offspring of mothers with type 2 diabetes mellitus treated with metformin throughout the pregnancy. Therefore, a separate study is needed to measure testis size in boys born to these mothers.

Maternal smoking during pregnancy has been associated with impaired reproductive development in male offspring, including reduced testicular size and semen quality, and increased risk of cryptorchidism [18, 19]. In our study, no adjustment for maternal smoking was needed, since there was no difference in the prevalence of maternal smoking between the metformin and insulin group ($p = 0.70$).

Only one boy out of 52 (1.9%) had a history of congenital cryptorchidism, which is in line with the previously described prevalence of this condition in the Turku area in Finland (2.4% at birth and 1.0% at the age of three months) [29]. Mild, diet-treated GDM has been shown to be associated with an increased risk of congenital cryptorchidism [16]. There is no information concerning the association of metformin and cryptorchidism. In the present study, one boy with congenital cryptorchidism was born to a mother treated with metformin during pregnancy. She also smoked, which has been associated with an additional increased risk of cryptorchidism in the offspring [18]. However, the sample size of our study is too small to estimate the possible connection between metformin and cryptorchidism.

Power calculations were performed in our primary RCT with respect to birth weight, using a non-inferiority design [2]. In the present testis study, a secondary power calculation was performed to determine the difference in testicular size (between boys born to mothers in the metformin and those born to mothers in the insulin group) which could be excluded with 80% power and significance level of 0.05 when the group size was 26 per group. This analysis revealed that our sample size was sufficiently large to exclude a 0.24 ml difference in testicular size determined by ultrasonography, i.e. we were able to exclude a difference between the treatments that is smaller than the SD of testicular size (0.3 ml). Moreover, all other testis size measurements were very similar in the metformin and insulin group, suggesting that any meaningful difference between the treatments can be excluded.

To our knowledge, this is the first study to evaluate the influence of metformin exposure on testicular development of human infants. The rate of participation in this follow-up study was nearly 50%, which we consider acceptable in view of the delicate nature of testis examination. Additionally, the sample size of the present study was sufficiently large at 52 infants. All three different methods applied to measure testicular size in this study are widely used, and the measurements were carried out by two trained professionals who were unaware of which treatment group the infants had been assigned to. Moreover, the results of all three methods were consistent; none of them detected any difference in testicular size between the two groups.

Regarding the representativeness of the data in this study, there was a modest difference of approximately two days in gestational weeks at birth between participating and non-participating boys from the original RCT [2]; there was no difference in other baseline variables. The difference in gestational weeks may be due to chance. It is unlikely that it could have affected our findings obtained from the comparison of testis size between the groups.

The present results alone are not sufficient to justify an alteration in the treatment policy of GDM. Studies on the long-term safety of the offspring are needed. They may include various physical and psychological aspects of health, and determine whether metformin could become the first-choice treatment in place of insulin in GDM. The present study suggests that metformin treatment of GDM is safe with respect to the reproductive health of male offspring.

5. Conclusions

Prenatal metformin exposure in male rodent offspring caused retarded growth of testes. Based on these observations, we performed a study comparing testicular size in 52 boys aged 33 to 85 months exposed to intrauterine metformin or insulin. The mothers had taken part in an earlier randomized controlled trial comparing metformin and insulin in the treatment of GDM. The testis measurements were performed with three different methods: ruler, orchidometer and ultrasonography. There was no difference in testicular size measured by any of the methods between metformin and insulin group boys. The results suggest that metformin is safe in the treatment of GDM in terms of the prepubertal gonad development of male offspring. However, further studies are needed to examine the possible association between intrauterine metformin exposure and testicular development, including hormonal functions, during puberty.

Acknowledgments: We thank biostatistician Tero Vahlberg for his kind help in statistical analyses. We also thank laboratory technician Mrs. Johanna Järvi for her help in data collection.

Funding: This study was financially supported by the Clinical Research (EVO) funding of Turku University Hospital, Academy of Finland, and Sigfrid Jusélius Foundation.

Disclosures: The authors report no conflict of interests.

■ References

1. **Rowan JA, Hague WM, Gao W, Battin MR, Moore MP, MiG Trial Investigators.** Metformin versus insulin for the treatment of gestational diabetes. *N Engl J Med* 2008. 358(19):2003-2015.
2. **Tertti K, Ekblad U, Koskinen P, Vahlberg T, Rönnemaa T.** Metformin vs. insulin in gestational diabetes. A randomized study characterizing metformin patients needing additional insulin. *Diabetes Obes Metab* 2013. 15(3):246-251.
3. **Mesdaghinia E, Samimi M, Homaei Z, Saberi F, Moosavi S, Yaribakht M.** Comparison of newborn outcomes in women with gestational diabetes mellitus treated with metformin of insulin: a randomized blinded trial. *Int J Prev Med* 2013. 4(3):327-333.
4. **Niromanesh S, Alavi A, Sharbaf F, Amjadi N, Moosavi S, Akbari S.** Metformin compared with insulin in the management of gestational diabetes mellitus: a randomized clinical trial. *Diabetes Res Clin Pract* 2012. 98(3):422-429.
5. **Ijäs H, Vääräsmäki M, Morin-Papunen L, Keravuo R, Ebeling T, Saarela T, Raudaskoski T.** Metformin should be considered in the treatment of gestational diabetes: a prospective randomized study. *BJOG* 2011. 118(7):880-885.
6. **Spaulonci C, Bernardes L, Trindade T, Zugaib M, Francisco R.** Randomized trial of metformin vs. insulin in the management of gestational diabetes. *Am J Obstet Gynecol* 2013. 209(1):34.
7. **Moore L, Briery C, Clokey D, Martin R, Williford N, Bofill J, Morrison J.** Metformin and insulin in the management of gestational diabetes mellitus: preliminary results of a comparison. *J Reprod Med* 2007. 52(11):1011-1015.
8. **Vanky E, Zahlsen K, Spigset O, Carlsen S.** Placental passage of metformin in women with polycystic ovary syndrome. *Fertil Steril* 2005. 83(5):1575-1578.
9. **Tertti K, Laine K, Ekblad U, Rinne V, Rönnemaa T.** The degree of fetal metformin exposure does not influence fetal outcome in gestational diabetes mellitus. *Acta Diabetol* 2014. 51(5):731-738.
10. **Glueck C, Goldenberg N, Pranikoff J, Loftspring M, Sieve L, Wang P.** Height, weight, and motor-social development during the first 18 months of life in 126 infants born to 109 mothers with polycystic ovary syndrome who conceived on and continued metformin through pregnancy. *Hum Reprod* 2004. 19(6):1323-1330.
11. **Ro T, Ludvigsen H, Carlsen S, Vanky E.** Growth, body composition and metabolic profile of 8-year-old children exposed to metformin in utero. *Scand J Clin Lab Invest* 2012. 72(7):570-575.
12. **Carlsen S, Martinussen M, Vanky E.** Metformin's effect on first-year weight gain: a follow-up study. *Pediatrics* 2012. 130(5):e1222-e1226.
13. **Rowan J, Rush E, Obolonkin V, Battin M, Wouldes T, Hague W.** Metformin in gestational diabetes: the offspring follow-up (MiG TOFU): body composition at 2 years of age. *Diabetes Care* 2011. 34(10):2279-2284.
14. **Ijäs H, Vääräsmäki M, Saarela T, Keravuo R, Rau-**

daskoski T. A follow-up of a randomised study of metformin and insulin in gestational diabetes mellitus: growth and development of the children at the age of 18 months. *BJOG* 2015. 122(7):994-1000.
15. **Tartarin P, Moison D, Guibert E, Dupont J, Habert R, Rouiller-Fabre V, Frydman N, Pozzi S, Frydman R, Lecureuil C, et al.** Metformin exposure affects human and mouse fetal testicular cells. *Hum Reprod* 2012. 27(11):3304-3314.
16. **Virtanen H, Tapanainen A, Kaleva M, Suomi A, Main K, Skakkebaek N, Toppari J.** Mild gestational diabetes as a risk factor for congenital cryptorchidism. *J Clin Endocrinol Metab* 2006. 91(12):4862-4865.
17. **Dinsdale H, Ridler C, Ells L.** A simple guide to classifying body mass index in children. *Oxford: National Obesity Observatory*, 2011.
18. **Hackshaw A, Rodeck C, Boniface S.** Maternal smoking in pregnancy and birth defects: a systematic review based on 173,687 malformed cases and 11.7 million controls. *Hum Reprod Update* 2011. 17(5):589-604.
19. **Jensen T, Jorgensen N, Punab M, Haugen T, Suominen J, Zilaitiene B, Horte A, Andersen A, Carlsen E, Magnus O, et al.** Association of in utero exposure to maternal smoking with reduced semen quality and testis size in adulthood: a cross-sectional study of 1,770 young men from the general population in five European countries. *Am J Epidemiol* 2004. 159(1):49-58.
20. **Wohlfahrt-Veje C, Tinggaard J, Winther K, Mouritsen A, Hagen C, Mieritz M, de Renzy-Martin K, Boas M, Petersen J, Main K.** Body fat throughout childhood in 2647 healthy Danish children: agreement of BMI, waist circumference, skinfolds with dual X-ray absorptiometry. *Eur J Clin Nutr* 2014. 68(6):664-670.
21. **Tomova A, Deepinder F, Robeva R, Lalabonova H, Kumanov P, Agarwal A.** Growth and development of male external genitalia: a cross-sectional study of 6200 males aged 0 to 19 years. *Arch Pediatr Adolesc Med* 2010. 164(12):1152-1157.
22. **Sotos J, Tokar N.** Testicular volumes revisited: a proposal for a simple clinical method that can closely match the volumes obtained by ultrasound and its clinical application. *Int J Pediatr Endocrinol* 2012. 2012(1):17.
23. **Goede J, Hack W, Sijstermans K, van der Voort-Doedensb L, Van der Ploegc T, Meij-de Vriesd A, Delemarre-van de Waal H.** Normative values for testicular volume measured by ultrasonography in a normal population from infancy to adolescence. *Horm Res Paediatr* 2011. 76(1):56-64.
24. **Salomäki H, Vähätalo L, Laurila K, Jäppinen N, Penttinen A, Ailanen L, Ilyasizadeh J, Pesonen U, Koulu M.** Prenatal metformin exposure in mice programs the metabolic phenotype of the offspring during a high fat diet at adulthood. *Plos One* 2013. 8(2):e56594.
25. **Salomäki H, Heinäniemi M, Vähätalo L, Ailanen L, Eerola K, Ruohonen S, Pesonen U, Koulu M.** Prenatal metformin exposure in a maternal hight fat diet mouse model alters the transcriptome and modifies the metabolic responses of the offspring. *Plos One* 2014. 9(12):e115798.
26. **Andersson A, Toppari J, Haavisto A, Petersen J, Simell T, Simell O, Skakkebaek N.** Longitudinal reproductive hormone profiles in infants: peak of inhibin B levels in infant boys exceeds levels in adult men. *J Clin Endocrinol Metab* 1998. 83(2):675-681.
27. **Kuiri-Hänninen T, Sankilampi U, Dunkel L.** Activation of the hypothalamic-pituitary-gonadal axis in infancy: minipuberty. *Horm Res Paediatr* 2014. 82(2):73-80.
28. **Carlsen S, Vanky E.** Metformin influence on hormone levels at birth, in PCOS mothers and their newborns. *Hum Reprod* 2010. 25(3):786-790.
29. **Boisen K, Kaleva M, Main K, Virtanen H, Haavisto A, Schmidt I, Chellakooty M, Damgaard I, Mau C, Reunanen M, et al.** Difference in prevalence of congenital cryptorchidism in infants between two Nordic countries. *Lancet* 2004. 363(9417):1264-1269.