RBMO

ELSEVIER

REVIEW

Higher live birth rate following transdermal testosterone pretreatment in poor responders: a systematic review and meta-analysis

BIOGRAPHY

Evangelia Katsika graduated from the Medical School of Aristotle University of Thessaloniki, Greece, in 2018 and received her Master's degree in Human Reproduction in 2022. She currently is an Obstetrics and Gynecology Resident at University College London Hospital, London, UK, with an interest in infertility and gynaecological endocrinology.

Evangelia T. Katsika[1], Julia K. Bosdou[1], Dimitrios G. Goulis[2], Grigoris F. Grimbizis[1,2], Efstratios M. Kolibianakis[1,*]

◇KEY MESSAGE

IVFに向けて卵巣刺激を施行しているPOR（poor ovarian responder）患者への経皮的テストステロン前処置は、生産率および臨床的妊娠率（以下、妊娠率）を上昇させる。

◇ABSTRACT

POR患者におけるテトステロン前処置の効果に関連する8個のRCT（n: 797人）を用いてsystematic review・メタ解析を施行。介入：テストステロンジェルの経皮的投与（用量：10-12.5 mg/日、投与期間：10–56日）。main outcome：生産率・妊娠率の著しい上昇。Secondary outcome：卵丘-卵母細胞複合体（COC）採取数の上昇、卵巣刺激期間の短縮、ゴナドトロピン製剤の使用量の減少、PORによるキャンセル率の低下、trigger投与日の子宮内膜厚の肥厚。エストラジオール（E2）濃度、17mm以上の卵胞数、第二減数分裂中期卵（MⅡ期卵）数、2前核期

胚（2PN 胚）数、移植胚数および胚移植をうけれた患者の割合については有意差なし。

◇INTRODUCTION

IVF にて卵巣刺激法を受ける患者の 9-24%は POR で、妊娠率は極めて低い。アンドロゲンは初期の卵胞発育を促し、原始卵胞・前胞状卵胞・胞状卵胞の数を増殖、卵巣の FSH への反応性を高める。

POR 患者におけるアンドロゲン補充療法についての RCT はいくつかあり、7 個の systematic review でメタ解析されているが、その効果について確立した結果は得られていない。最近では Neves らが systematic review にてテストステロン前処置による生産率の上昇を報告しているが、文献の収集に不十分・不適切なところがあり、更にはテストステロン前処置および卵巣刺激期の投与も区別されていない。

本報告では、IVF の為の卵巣刺激を施行中の POR 患者におけるテストステロン前処置と妊娠について、妊娠率および生産率を用いて評価した。

◇MATERIALS AND METHODS

本報告は PROSPERO に 2021/8/22 に登録（No.CRD42021262098）。

Search strategy（TABLE1）

・データベース：MEDLINE、EMBASE、CENTRAL、ISI Web of Science、SCOPUS

・期間：2022/7 まで

・reviewer：2 人（E.T.K.、J.K.B.）

・対象文献：RCT（IVF に向けて GnRH アナログ製剤およびゴナドトロピン製剤にて卵巣刺激を施行している POR 患者においてテストステロン前処置が妊娠率を上昇させるかについて評価しているもの）

Selection of studies（TABLE2）

▪ Include：

ⅰ)対象：POR 患者（定義不問）

ⅱ)介入群：テストステロン前処置施行（用量・治療期間不問）

ⅲ)卵巣刺激：ゴナドトロピン製剤+GnRH アナログ製剤使用

ⅳ)対照群：介入群と同じデザインで割付されている

▪ Exclude：TABLE2 参照

Date extraction（TABLE3＆4）

Assessment of risk of bias

リスクオブバイアスの評価：GRADEproGDT ソフトウェアを用いた GRADE アプローチにて評価

Outcomes

▪ RESULT の解釈：ITT 解析使用

▪ Main outcome: 妊娠率（妊娠 6－8 週時に子宮腔内の GS・FHM 確認）もしくは生産率

▪ Secondary outcome: ゴナドトロピン製剤の使用量・期間、E2 濃度、trigger 投与日の子宮内膜厚・17mm 以上の卵胞数、POR によるキャンセル率、COC 採取数、移植胚数、MⅡ期卵数、2PN 胚数、流産率、胚移植をうけた患者の割合

▪ 欠損データは研究著者に確認。

Quantitative data synthesis

2 値 outcome: FEM/Mantel-Haenszel アプローチ、REM/ DerSimonian and Laird アプローチを用いて、RR(95%CI)にて評価。連続値 outocome：FEM/inverse variance method、REM/ DerSimonian and Laird method を用いて、WMD(95%CI)にて評価。Results：STATA/MP

Softwareを用いてメタ解析に組み込まれた。研究間のばらつき：カイ二乗検定にて評価。FEM:異質性が低い際(I^2<40%)に使用。REM:異質性が高い際に使用。有意水準：p<0.05。出版バイアス：Harbord-Egger's 検定にて評価。

Qualitative data synthesis

コクランリスクオブバイアス 2(RoB2)使用

◇RESULTS

Identification of literature(FIGURE1)

2012件の文献の内、801件は重複、1194件はtitleおよびabstract不適、9件は介入が非対称にて除外。最終的に8件の文献を組み入れた。

Systematic review(TABLE3&4)

▪ TABLE3：review対象は8件のRCT（2006-2021年、合計797人：50-132人/RCT)。ランダム化方法報告 6/8、割り当ての隠蔽 3/8、PORの定義及びprimary outcomeは様々。Power analysis3/8。Financial support3/8。

▪ TABLE4：早発LHサージ対策(GnRHアンタゴニスト 6/8、GnRHアゴニスト 1/8、両方 1/8)、卵巣刺激(rFSH5/8、hMG1/8、rFSH+hMG1/8)、ゴナドトロピン製剤の調節 5/8、trigger(HCG8/8)、HCGの投与時期・用量は様々、採卵時期(trigger投与から35-40時間後 8/8：36時間後 4/8)、IVF 2/8・ICSI 3/8・IVF/ICSI 3/8、胚移植時期(採卵後 Day 2or3)、黄体補充療法様々、テストステロンジェルによる重大な副作用なし。

Intervention(TABLE5)

経皮的テストステロン前処置(ジェル、10-12.5 mg/日、10-56日間)

Risk of bias assessment results

Rob2 による評価では、low-risk3/8、some concerns3/8、high-risk2/8。

Meta-analysis

- **Primary outcome(FIGURE2＆3)**

生産率上昇(RR 2.07、95%CI 1.09-3.92；RD 10%、95%CI 2-17；FEM I^2 0%；4 文献、333 人)、妊娠率上昇(RR 2.25、95%CI 1.54-3.30；RD 11%、95%CI 4-18；FEM I^2 0%；8 文献、797 人)

- **Secondary outcomes that improved following testosterone pretreatment(TABLE6)**

卵巣刺激期間短縮(WMD −0.81)、ゴナドトロピン製剤の使用量減少(WMD −368.8IU)、trigger 投与日の子宮内膜厚肥厚(WMD 0.83mm)、POR によるキャンセル率低下(RR 0.37)、COC 採取数増加(WMD 0.88)

- **Secondary outcomes that not significantly different following testosterone pretreatment(TABLE6)**

trigger 投与日の E2 濃度(WMD −8.12pg/ml)、trigger 投与日の 17mm 以上の卵胞数(WMD 0.82)、MⅡ期卵数(WMD 0.48)、2PN 胚数(WMD 0.49)、移植胚数(WMD 0.21)、流産率(RR 1.12)、胚移植をうけた患者の割合(RR 1.00)

◇DISCUSSION

本解析により、経皮的テストステロン前処置が POR 患者の IVF における妊娠率を上昇させることが示唆された。COC 採取量が増加したことが生産率・妊娠率の上昇につながったと考えられた。テストステロンおよび DHT はアンドロゲンの中で唯一生物学的活性を有し、直接受容体に結合できる。霊長類における in vivo 実験では、テストステロン・DHT

が前胞状卵胞・胞状卵胞を増加させ、卵胞の発育を促進するということが判明している。テストステロン・DHTは、IGF-1およびその受容体の発現を増幅させる。IGF-1は顆粒膜・莢膜・間質細胞・卵母細胞に存在しており、卵胞のアポトーシスを減じる。そのため、テストステロンはIGF-1を介して卵母細胞や胚の質に影響を与えることができる。更に、アンドロゲンRおよびFSHRは相互作用しており（テストステロン⇒FSHR発現増加、FSH⇒アンドロゲンR発現増加）、アンドロゲンは卵胞閉鎖や顆粒膜細胞のアポトーシスを減らし、その増殖・分化を促進するため、卵胞維持のためにはかかせない存在である。

本reviewは、今までの類似reviewよりも規模がかなり大きく、組み込み・除外項目も厳しく設定しており、より正確な評価、結果につながった。また、経皮的テストステロン前処置の副作用は塗布部位の掻痒感1例のみで、治療の安全性も示された。しかし、長期投与による影響や、出生後の児のデータなどは未評価。更に、Limitationとして、PORの定義やテストステロンの投与量・投与期間に統一性がなかったことが挙げられ、更なる研究が求められる。

TABLE 1 SEARCH TERMS USED FOR THE IDENTIFICATION OF ELIGIBLE STUDIES

Intervention	Population	Setting
(testosteron*)	AND [(poor) OR (low) OR (slow) OR (inadequate) OR (suboptimal) OR (decreas*) OR (diminish*)] AND (respon*) OR (reserve)	(in-vitro fertilization) OR (in vitro fertilization) OR (IVF) OR (intracytoplasmic sperm injection) OR (ICSI) OR (IVF/ICSI)

TABLE 2 EXCLUDED STUDIES AND REASONS FOR EXCLUSION

Study	Reason for exclusion
Sipe et al. (1986)	Case report
Balasch et al. (2006)	Prospective, self-controlled trial
Fábregues et al. (2009)	Co-intervention
Sipe et al. (2010)	Prospective, randomized, crossover trial
Saharkhiz et al. (2018)	Testosterone administration during ovarian stimulation
Hassan et al. (2019)	Crossover trial
Erin Ahart et al. (2019)	Prospective, cohort, non-randomized trial
Padmashri et al. (2019)	Not performed in poor responders
Andreeva et al. (2020)	Observational, pilot study

TABLE 3 METHODOLOGICAL CHARACTERISTICS OF ELIGIBLE RCT

Authors (year), journal	ITT (n)	Per protocol (n)	Testosterone pre-treatment	No pre-treatment	Definition of poor ovarian response	Randomization method	Blinding	Primary outcome	Financial support
Massin et al. (2006), *Human Reproduction*	53	49	24	25	Oestradiol <1200 pg/ml on day of HCG and ≤ 5 COC retrieved, and day 3 FSH >12 IU/l, oestradiol >70 pg/ml and inhibin B <45 pg/ml	Computer-generated randomization list	Double blind	COC retrieved	Yes
Kim et al. (2011), *Fertility and Sterility*	110	110	55	55	≤3 COC retrieved despite the use of a high total gonadotrophin dose (>2500 IU)	Computer-generated randomization list	No	Mature oocytes retrieved	Not reported
Kim et al. (2014), *Development and Reproduction*	120	120	30, 30, 30[a]	30	≤3 COC retrieved despite the use of a high total gonadotrophin dose (>2500 IU)	Computer-generated randomization list	No	Mature oocytes retrieved	Not reported
Bosdou et al. (2016), *Human Reproduction*	50	50	26	24	Bologna criteria	Computer-generated randomization list	Single blind	COC retrieved	Partially funded by a scholarship
Doan et al. (2017), *Gynecological Endocrinology*	110	110	55	55	AFC <5-7 follicles or AMH ≤1.26 ng/ml	Not reported	Not reported	Not reported	Not reported
Al-Jeborry (2019), *Annals of Tropical Medicine and Public Health*	132	132	71	61	POSEIDON criteria	Not reported	Not reported	Number of retrieved and mature oocytes and pregnancy rate	No
Hoang et al. (2021), *Reproductive Medicine and Biology*	159	122	42, 38[a]	42	Bologna criteria	Manual lottery	Single	Total number of retrieved mature oocytes	Not reported
Subirá et al. (2021), *Reproductive BioMedicine Online*	63	49	17, 16 [a]	16	Bologna criteria	Computer-generated randomization list	Single	Mature oocytes retrieved	Yes

[a] Testosterone was applied for different durations in different groups of patients.

AFC, antral follicle count; AMH, anti-Mullerian hormone; COC, cumulus-oocyte complex; HCG, human chorionic gonadotrophin; ITT, intention-to-treat; RCT, randomized controlled trial.

TABLE 4 CYCLE CHARACTERISTICS OF ELIGIBLE RCT

Authors (year)	GnRH analogue	Type of analogue protocol	Gonadotrophin type/ starting dose	Gonadotrophin adjustments	Signal for triggering oocyte maturation	Criteria for HCG administration	OPU	Fertilization	Embryo transfer	Type of luteal support	Embryo quality studied	Adverse effects
Massin et al. (2006)	Triptorelin or cetrorelix	Mainly long GnRH agonist but also short GnRH agonist and antagonist (proportions of GnRH analogue protocols were not statistically different between the two arms)	rFSH/300-450 IU	Yes, no further information provided	10,000 IU uHCG	At least three follicles 17 mm in diameter	36 h after HCG	IVF/ICSI	Day 2/3	Micronized progesterone 200 mg/b.i.d. vaginally and 2500 IU HCG at 3, 6 and 9 days after HCG for triggering final oocyte maturation	Not reported	None
Kim et al. (2011)	Cetrorelix 0.25 mg/day	Flexible protocol (when lead follicle reached 13-14 mm in diameter)	r(h)FSH/300IU	Yes, every 3-4 days according to ovarian response	250 mg rHCG	At least one follicle of at least 18 mm in diameter	36 h after HCG	IVF/ICSI	Day 3	Vaginal progesterone gel 90 mg/day	Number of grade 1 and 2 embryos	None
Kim et al. (2014)	Cetrorelix 0.25 mg/day	Flexible protocol (when lead follicle reached 13-14 mm in diameter)	r(h)FSH/300 IU	Yes, every 3-4 days according to ovarian response	250 mg rHCG	At least one follicle of at least 18 mm in diameter	35-36 h after HCG	IVF/ICSI	Day 3	Vaginal progesterone gel 90 mg/day	Number of grade 1 and 2 embryos	None
Bosdou et al. (2016)	Triptorelin 3.75 mg depot, followed by daily injections of triptorelin 0.1 mg, if necessary	Long GnRH agonist protocol	Follitropin alpha/-	Not reported	250 mg rHCG	At least two follicles reached 17 mm in diameter or, if this was not possible, the maximum number of follicles were present	36 h after HCG	ICSI	Day 2	Vaginal micronized progesterone 600 mg/day	Quality of embryos on day 2 of in-vitro culture (top/medium/low)	None
Doan et al. (2017)	Not reported	GnRH antagonist protocol	Not reported	Not reported	5000 or 10,000 IU HCG	At least two follicles of more than 17 mm size	35-40 h after HCG	IVF	Not reported	Not reported	Not reported	Not reported
Al-Jeborry et al. (2019)	Cetrotide 0.25 mg/day	Flexible protocol (when lead follicle reached 13-14 mm in diameter)	r(h)FSH/300-4500 IU with or without addition of Menogon 75-150 IU daily	Yes, every few days according to ovarian response/ measuring serial serum oestradiol levels	5000-10,000 IU uHCG	Two or three follicles more than 17-18 mm size	34-35 h after HCG	ICSI	Day 3	Cyclogest progesterone suppository vaginally in dose of 400 mg twice daily	Not reported	Not reported
Hoang et al. (2021)	Cetrotide 0.25 mg/day	GnRH antagonist protocol	rFSH/300 IU	Yes, based on patient's condition and individual ovarian response	6500 IU HCG	At least two follicles reached at least 18 mm	36-37 h after HCG	IVF	Day 3	Micronized progesterone 800 mg/day	Number of good-quality embryos according to Istanbul consensus	Light itching
Subirá et al. (2021)	Not reported	Conventional short antagonist protocol	Human menopausal gonadotrophin/300 IU	No	Not reported	At least one follicle measured over 16 mm	36 h after HCG	ICSI	Day 2 or 3	Not reported	Number of day 3 embryos	None

b.i.d., twice daily; GnRH, gonadotrophin-releasing hormone; HCG, human chorionic gonadotrophin; ICSI, intracytoplasmic sperm injection; OPU, Oocyte pick up; RCT, randomized controlled trials; rFSH, recombinant FSH; r(h)FSH, recombinant (human) FSH; rHCG, recombinant human chorionic gonadotrophin; uHCG, urinary human chorionic gonadotrophin.

TABLE 5 PROTOCOLS USED FOR TESTOSTERONE PRETREATMENT

Authors (year), journal	Testosterone administration			
	Type	Route	Dose	Duration
Massin et al. (2006), Human Reproduction	Testosterone gel 1%	Transdermal	10 mg/day	15–20 days
Kim et al. (2011), Fertility and Sterility	Testosterone gel 1%	Transdermal	12.5 mg/day	21 days
Kim et al. (2014), Development and Reproduction	Testosterone gel 1%	Transdermal	12.5 mg/day	Subgroup a: 14 days Subgroup b: 21 days Subgroup c: 28 days
Bosdou et al. (2016), Human Reproduction	Testosterone gel 1%	Transdermal	10 mg/day	21 days
Doan et al. (2017), Gynecological Endocrinology	Testosterone gel 1%	Transdermal	12.5 mg/day	From day 6 of previous menstrual period to day 2 of stimulated menstrual period
Al Jaberry (2019), Annals of Tropical Medicine and Public Health	Testosterone gel 1%	Transdermal	10 mg/day	21 days
Hoang et al. (2021), Reproductive Medicine and Biology	Testosterone gel 1%	Transdermal	12.5 mg/day	Subgroup a: 28 days Subgroup b: 42 days
Subirá et al. (2021), Reproductive BioMedicine Online	Testosterone gel 1%	Transdermal	12.5 mg/day	Subgroup a (long testosterone): 56 days Subgroup b (short testosterone): 10 days

TABLE 6 SECONDARY OUTCOMES FOLLOWING TESTOSTERONE PRETREATMENT

	Studies	Sample size	Method applied	Effect(95% CI)
Outcomes improved following testosterone pretreatment				
Duration of ovarian stimulation (days)	7	744	Random effects model, I^2: 92%	WMD: −0.81 (−1.46 to −0.16)
Total dose of gonadotrophins required for ovarian stimulation (IU)	8	797	Random effects model, I^2: 87%	WMD: −369.8 (−612.4 to −125.2)
Endometrial thickness on the day of triggering final oocyte maturation (mm)	5	561	Random effects model, I^2: 77.6%	WMD: 0.83 (0.13 to 1.53)
Cancellation rate due to poor ovarian response	6	681	Fixed effects model, I^2: 0%	RR: 0.37 (0.20 to 0.71)
No. of cumulus–oocyte complexes retrieved	8	797	Random effects model, I^2: 78.7%	WMD: 0.88 (0.22 to 1.54)
Outcomes not significantly different following testosterone pretreatment				
Oestradiol concentration on the day of triggering final oocyte maturation (pg/ml)	4	394	Fixed effects model, I^2: 0%	WMD: −8.12 (−118.2 to 101.96)
No. of follicles ≥17 mm on the day of triggering final oocyte maturation	5	386	Random effects model, I^2: 85.7%	WMD: 0.82 (−0.11 to 1.74)
No. of metaphase II oocytes	3	245	Fixed effects model, I^2: 0%	WMD: 0.48(−0.16 to 1.13)
No. of 2pn oocytes	7	665	Random effects model, I^2: 80.3%	WMD: 0.49 (−0.11 to 1.10)
No. of embryos transferred	8	797	Random effects model, I^2: 70.5%	WMD: 0.21 (−0.07 to 0.49)
No. of miscarriages	3	202	Fixed effects model, I^2: 0%	RR: 1.12 (0.30 to 4.22)
Proportion of patients having an embryo transfer	8	797	Fixed effects model, I^2: 15.9%	RR: 1.00 (0.96 to 1.04)

2pn, two pronuclear; CI, confidence interval; I^2, heterogeneity; RR, risk ratio; WMD, weighted mean difference.

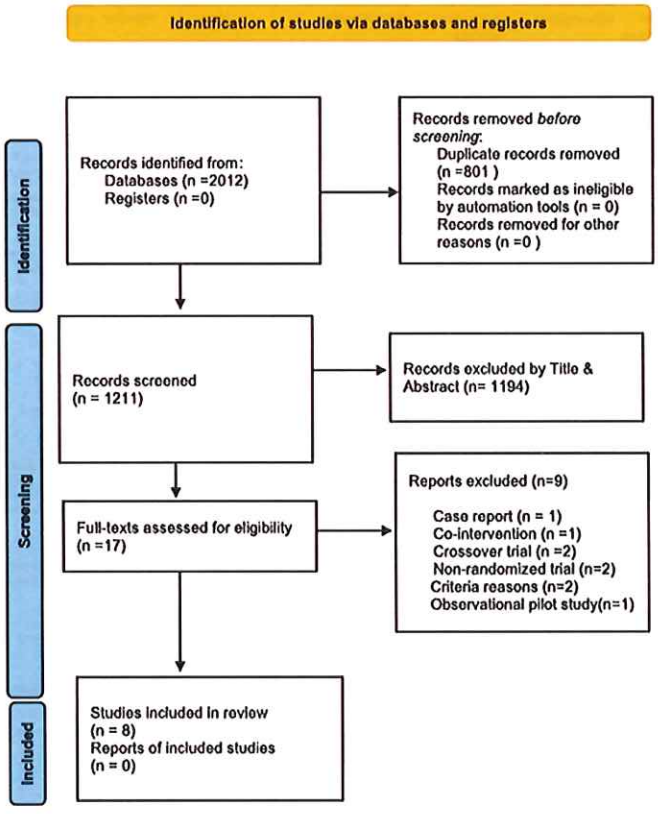


FIGURE 1 PRISMA flow diagram detailing the selection of studies for inclusion.

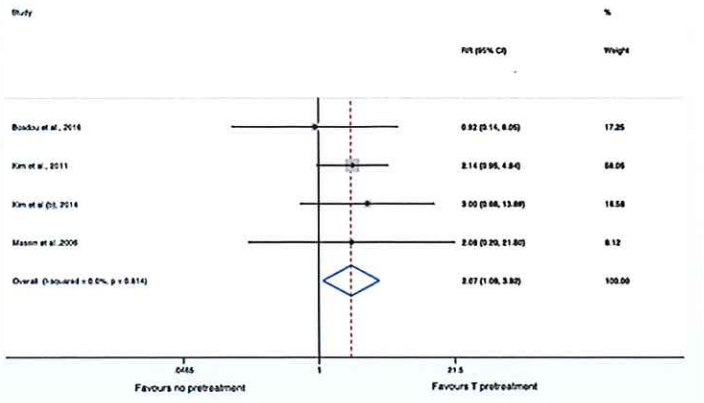


FIGURE 2 Risk ratio (RR) with 95% confidence interval (CI) for live birth in patients with poor ovarian response pretreated or not pretreated with testosterone (T). When the studies were evaluating testosterone pretreatments of different durations, subgroups a–c were created in each study for more precise results. Therefore, a, b or c in brackets next to the study authors represents the particular subgroup from the total sample size evaluating testosterone pretreatment of different durations.

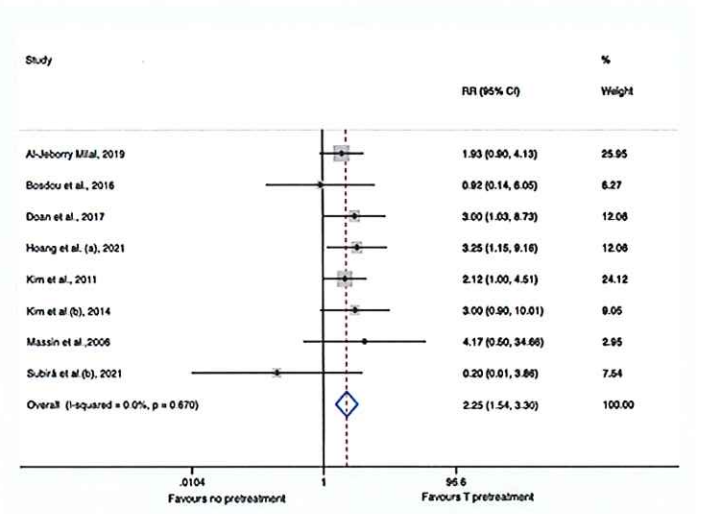
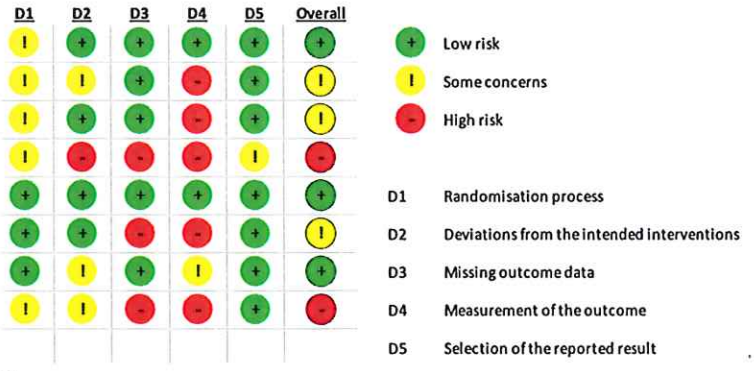


FIGURE 3 Risk ratio (RR) with 95% confidence interval (CI) for clinical pregnancy in patients with poor ovarian response pre-treated or not pretreated with testosterone (T). When the studies were evaluating testosterone pretreatments of different durations, subgroups a–c were created in each study for more precise results. Therefore, a, b or c in brackets next to the study authors represents the particular subgroup from the total sample size evaluating testosterone pretreatment of different durations.



Supplementary Figure I. The assessment of methodological quality and the risk of bias of the included studies using the Risk of Bias (RoB) 2 tool