Bilirubin in follicular fluid: a biochemical signature of female infertility

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INTRODUCTION

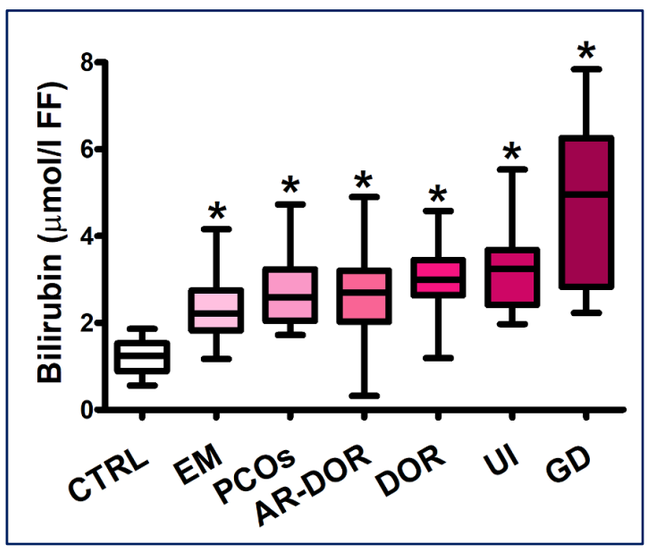
Successful in vitro fertilization (IVF) are unexplainably low (about 32%), not only in females with full-blown pathologies, but also in those known as idiopathic. Studies indicated that the composition of follicular fluid (FF) of infertile female may show altered levels of proteins, lipids, hormones respect to values found in fertile females. Such changes were associated to endometriosis (EM), polycystic ovarian syndrome (PCOs), diminished ovarian reserve (DOR). To date, no studies have been dedicated either to determine potential differences in the FF concentration of bilirubin in fertile and infertile females or to evaluate possible correlations with main IVF outcome measures.

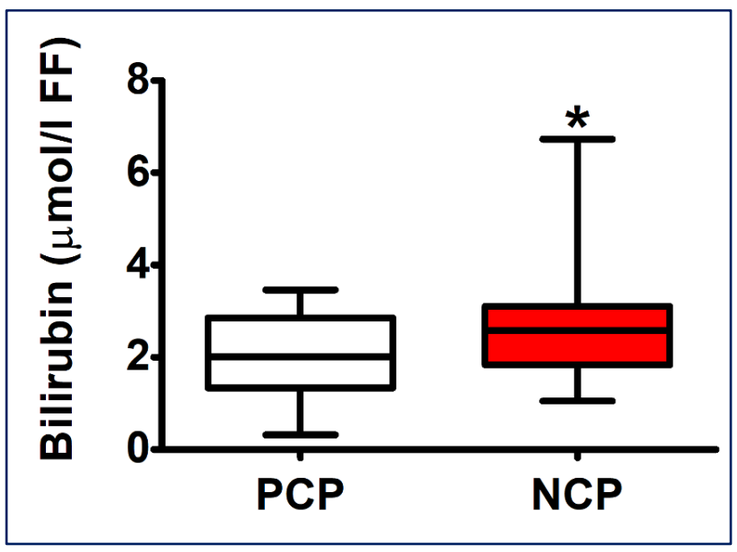
STUDY DESIGN

Cross-sectional study in seven female groups: 1) controls (CTRL, n = 34); 2) EM (n = 19); 3) PCOs (n = 12); 4) age-related DOR (AR-DOR, n = 55); 5) DOR (n = 31); 6) unexplained infertility (UI, n = 16); 7) genetic disorders (GD, n = 11). Patients were enrolled from September 2018 to January 2020. As inclusion criteria, patients should have total plasma bilirubin within normal physiological range (3.4-17.1 μmol/l). FF was processed by high performance liquid chromatography (HPLC) to quantify bilirubin concentration. For all patients were counted the number of cumulus-oocyte complexes (COCs), MII oocytes, zygotes and blastocysts.

RESULTS

FF total bilirubin concentration (Fig. 1) was 1.20 ± 0.42 μmol/l in controls (n = 34) whereas total bilirubin in patients with EM, PCOs, AR-DOR, DOR, UI and GD was, respectively 2.34 ± 0.80, 2.74 ± 0.84, 2.66 ± 0.93, 2.99 ± 0.74, 3.18 ± 0.90, 4.77 ± 1.90 μmol/l FF (values of all groups were significantly different from those of controls, $p < 0.001$). To evaluate whether increase in FF total bilirubin might affect IVF outcome, we pooled all patients into a single group (n = 178) and calculated the Pearson's correlation coefficients of bilirubin (y) and number of MII oocytes (fx); bilirubin (y) and number of zygotes (fx); bilirubin (y) and number of good quality blastocysts (fx). Increase of FF total bilirubin negatively correlated with the number either of mature oocytes ($r = -0.348$, $t = -4.876$, $p < 0.001$), or zygotes ($r = -0.247$, $t = -2.959$, $p < 0.005$), or blastocysts ($r = -0.251$, $t = -3.033$, $p < 0.005$). As it could be expected chemical pregnancy rate and clinical pregnancy rate of each group of infertile females were significantly lower when compared to the corresponding values recorded in the group of control fertile females ($p < 0.05$).
Additionally, we categorized the single group of 178 patients into those with positive clinical pregnancy (PCP, n = 27) and those with negative clinical pregnancy (NCP, n = 101). Bilirubin in FF of these groups (Fig. 2) was 2.61 ± 1.05 and 2.03 ± 1.00 μmol/l ($p < 0.01$) confirming that probability of clinical pregnancy decreases in presence of higher bilirubin values.





CONCLUSION

If reinforced by the data of newborns, findings of the present study might represent a significant advancement in IVF treatment. Measuring total bilirubin in FF might be helpful in selecting the best oocytes for IVF/ICSI procedures, thereby possibly leading to a significant increase of IVF success rate.