Absence of a correlation between intra-follicular androgen and AMH levels and the oocyte count in poor responder patients undergoing in vitro fertilization

K Mutia1*, N Muna1, N M D Suratih2, R R Febri1, O Riayati1, R Muharam1,3,4, A Hestiantoro1,3,4, P A Iffanolida1 and B Wiweko1,3,4

1Human Reproductive, Infertility and Family Planning Research Center, Indonesia Medical Education and Research Institute (IMERI), Faculty of Medicine, Universitas Indonesia, Jakarta, 10430, Indonesia
2Department of Obstetrics & Gynecology Persahabatan Hospital, Persahabatan Public Hospital, Jakarta, 13230, Indonesia
3Division of Reproductive Endocrinology and Infertility Department of Obstetrics and Gynecology Faculty of Medicine Universitas Indonesia, Jakarta, 10430, Indonesia
4Yasmin IVF Clinic, Dr. Cipto Mangunkusumo General Hospital, Jakarta, 10430, Indonesia

*E-mail: budi.wiweko@gmail.com

Abstract. Androgens are male hormones and precursors of female hormones. Testosterone (T), dehydroepiandrostenedione (DHEA), and anti-mullerian hormone (AMH) enhance follicular recruitment and promote follicular growth and development. Clinical reports have demonstrated that co-treatment with androgens, such as DHEA and Androderm (transdermal testosterone) may increase both quantity and quality of oocytes and embryos and improve pregnancy outcomes in women with diminished ovarian function or premature ovarian failure. This study was designed to find correlations between intra-follicular androgen and AMH levels and the amount of oocytes retrieved from ovarian responders and poor-responders. We enrolled 40 patients undergoing in vitro fertilization (IVF) and tested their follicular fluid for testosterone, DHEA, and AMH levels. AMH levels were significantly different between the groups. However, no correlations were observed between the levels of testosterone, DHEA, or AMH and the amount of oocytes retrieved. No correlations were observed between androgens and AMH levels with the amount of oocytes in either group. Thus, there is no correlation between intra-follicular androgen and AMH levels and the amount of oocytes in both responders and poor responders. A larger sample size and multi-center study design is needed to confirm this.

1. Introduction

Infertility rates are increasing annually, and in Indonesia, approximately 10.2%–15.9% of couples are affected. The number of women classified as poor-responders based on ovarian response to conventional inducing agents has increased due to the global tendency to delay attempts to conceive. The rate of poor-responders during assisted reproductive technology (ART) vary between 9% and 24% [1]. In Indonesia, based on data from PERFITRI (2013–2017), the number of IVF cycles for patients aged above 40 years increased from 12.6% to 13.15% [2].

Content from this work may be used under the terms of the Creative Commons Attribution 3.0 licence. Any further distribution of this work must maintain attribution to the author(s) and the title of the work, journal citation and DOI.

Published under licence by IOP Publishing Ltd
The ART success rate decreases with increased age in women and is only approximately 8%–13% in women aged over 40 years. Likewise, women aged over 40 years have low numbers of oocytes and embryos, high embryo fragmentation rates, and lower implantation rates [2].

Poor responders were defined based on the Bologna criteria (with at least two of the criteria needed for diagnosis). The criteria were as follows: 1) advanced age (age, ≥40 years) or presence of other risk factors, 2) history of poor response on previous cycles with <3 oocytes retrieved after conventional protocol, and 3) poor ovarian reserve [antral follicle counts (AFCs) <5–7 follicles or AMH level <0.5–1.1 ng/ml) [3].

Androgens are important in folliculogenesis and follicular maturation in the ovaries [4-6]. Based on this, androgen levels (testosterone, DHEA, and androstenedione) have been investigated in late follicular fluids for associations with fertilization rates generated after stimulation [7].

However, the small number of cases studied has given contradictory results, with some indicating an association and others discarding it. In addition, no studies have focused on the three types of androgens and the fertilization rates in both poor- and normal-response patients, and the specific influence of androgens on the oocyte count and quality is unknown. This study was designed to detect associations between the intrafollicular androgen levels and the amounts of retrieved oocytes in poor-response patients.

2. Methods
This cross sectional study was conducted to find correlations between the AMH and androgen levels in follicular fluid with the amount of oocytes in ovarian responders and poor-responders. The study protocol was approved by the Health Research Ethics Committee, Faculty of Medicine, Universitas Indonesia-Cipto Mangunkusumo Hospital. Data was analyzed from 40 patients undergoing IVF cycles at the Yasmin Clinic, Cipto Mangunkusumo Hospital. The study was conducted between March 2017 and January 2018.

The patients were divided into two groups: poor responders and responders according to the Bologna criteria. Follicular fluid samples collected during the oocyte retrieval procedure were used. The measurement included the testosterone, DHEA, and AMH levels in follicular fluid using enzyme-linked immunosorbent assay (ELISA). Tools used in this study included the TOSOH kit (ST AIA-Pack DHEA-S, ST AIA-Pack Testosterone, TOSOH, Japan) for detecting testosterone and DHEA and a Beckman Coulter kit (AMH Gen II ELISA, Beckman Coulter, Brea, CA, USA) to quantify AMH levels. The correlations between Androgen (T and DHEA) and AMH levels and the amount of oocytes were analyzed using the SPSS statistical analysis software.

3. Results
Table 1 shows that the average age of the patients was 31 years for the responders and 37 years for the poor responders. A significant difference in age between the responders and poor responders was found (p = 0.003).

| Table 1. Subject Characteristics |
|---------------------------------|
| Normal-responders (n = 20)      | Poor-responders (n = 20) | p    |
| Age                             | 31.64 ± 1.1               | 37.30 ± 1.8 | 0.03 |
| Oocyte count                    | 11.15 ± 1.5               | 5.85 ± 0.8 | 0.7  |
| Testosterone (ng/dl)            | 10.7 ± 2.5                | 12.1 ± 2.5 | 0.2  |
| DHEA                            | 140.25 ± 75.6             | 154 ± 93.2 | 0.5  |
| AMH                             | 2.6 ± 2.5                 | 2.19 ± 3.0 | 0.05 |
The testosterone and DHEA levels were higher in poor responders than in non-poor responders. Further, the total amounts of oocytes and AMH levels were lower in poor-responders than in non-poor responder. However, the difference was only significant for the AMH level (p = 0.05).

**Table 2.** Correlation between Androgen and AMH Levels and the Oocyte Counts

|            | Mature oocytes |
|------------|----------------|
| Testosterone | r = 0.23       |
|            | p = 0.88       |
|            | n = 20         |
| DHEA       | r = 0.49       |
|            | p = 0.76       |
|            | n = 20         |
| AMH        | r = 0.17       |
|            | p = 0.26       |
|            | n = 20         |

The data presented in Table 2 indicates no correlations between testosterone, DHEA, and AMH levels in follicular fluid and the mature oocyte counts.

4. Discussion
Androgens are correlated with the fertilization rate, because the aromatization of androstenedione—facilitated by testosterone—to estrone is an important folliculogenesis step wherein both steroids need to be balanced. Higher or lower levels of androgens may impair estrogen production or may disturb follicle development and maturation. However, these mechanisms have not yet been well studied, probably because of the multiplicity of factors influencing follicle growth.

A study conducted by Revelli et al. (2009) showed that an increased testosterone level in follicular fluid is associated with oocyte quality and lower cleavage rates. Testosterone level may also be associated with early follicular atresia affecting oocyte viability and limiting fertilization and pregnancy. Although a theory regarding the intra-follicular environment causing follicular atresia exists, the intrafollicular androgen levels are required for optimal follicular development [8].

These results corroborate with those of Wen et al. (2010), who showed that androgen levels in follicular fluid were lower in fertilized oocytes [9]. However, in a study conducted by Lamb et al. (2010), the testosterone level in follicular fluid was significantly higher in fertilized oocytes than in degenerated oocytes [10].

Furthermore, De Los Santos et al. (2013) reported that testosterone levels were similar in women aged under 35 years and over 35 years, even though the androstenedione levels were lower in women aged over 35 years; however, this finding was not statistically significant [11]. In the present study, we also found no significant difference between the two groups.

Androgens have been proposed to work in combination with FSH in granulosa cells, and a study has shown a change in androgen metabolism with follicle maturation [12]. This finding is corroborated by those of Prizant, Gleicher, and Sen in 2014, who also conducted an overview of the effects of androgen on the ovaries. Another study has shown that androgen levels are associated with poor reproductive health. However, androgens play a major role in follicular development and maturity in the pre-antral phase and are also precursors of steroidogenesis in the final phase of folliculogenesis. Androgens bind to androgen receptors and work toward preventing follicular atresia. The number of androgen receptors decreases with the maturation of the follicle [13].
Lamb et al. (2010) assessed the association between hormonal levels in follicular fluid in patients after ovarian stimulation. He observed that intra follicular testosterone levels were higher in patients with good fertilization rates [10].

This study has some limitations. First, we did not consider several confounding factors, such as the endometriosis factor, that could have impacted our results. Second, in the present study, the actual baseline androgen levels could not be evaluated, because the androgen levels were measured after the ovarian stimulation protocol. The small sample size and singular place of recruitment may also have impacted the results.

5. Conclusion
This study found no correlation between the intra-follicular androgen and AMH levels in follicular fluid and the oocyte counts in either poor responders or non-poor responders. However, a multi-center study with a larger sample size and a thorough analysis of other confounding is warranted to further confirm the current findings.

References
[1] Mascarenhas M N, Flaxman R, Boerma T, Vanderpoel S and Stevens GA 2012 National, regional, and global trends in infertility prevalence since 1990: a systematic analysis of 277 health surveys PLoS Med. 9 e1001356
[2] PERFITRI. in Perhimpunan Fertilisasi In Vitro Indonesia (IAIVF, 2018).
[3] Ferraretti A, La Marca A, Fauser B C, Tarlatzis B, Nargund G, Gianaroli L and ESHRE working group on Poor Ovarian Response Definition 2011 ESHRE consensus on the definition of 'poor response' to ovarian stimulation for in vitro fertilization: the Bologna criteria Hum. Reprod. 26 1616–24
[4] Drummond A E 2006 The role of steroids in follicular growth Reprod. Biol. Endocrinol. 4 16
[5] Hillier S G, Whitelaw P F and Smyth C D 1994 Follicular oestrogen synthesis: the 'two-cell, two-gonadotrophin' model revisited Mol. Cell. Endocrinol. 100 51–4
[6] Sunkara S K and Coomarasamy A 2011 Androgen pretreatment in poor responders undergoing controlled ovarian stimulation and in vitro fertilization treatment Fertil. Steril. 95 e73–4
[7] Schünemann H J, Woodhead M, Anzuet A, Buist S, MacNee W, Rabe KF and Heffner J 2009 A vision statement on guideline development for respiratory disease: the example of COPD Lancet. 373 774–9
[8] Smitz J, Andersen A N, Devroep Y, Arce J C and Group M 2007 Endocrine profile in serum and follicular fluid differs after ovarian stimulation with HP-hMG or recombinant FSH in IVF patients Hum. Reprod. 22 676–87
[9] Wen X, Li D, Tozer A J, Docherty SM and Iles R K 2010 Estradiol, progesterone, testosterone profiles in human follicular fluid and cultured granulosa cells from luteinized pre-ovulatory follicles Reprod. Biol. Endocrinol. 8 117
[10] Lamb J D, Zamah AM, Shen S, McCulloch C, Cedars M I and Rosen M P 2010 Follicular fluid steroid hormone levels are associated with fertilization outcome after intracytoplasmic sperm injection Fertil. Steril. 94 952–7
[11] De los Santos M J, Garcia-Laez V, Beltrán D, Labarta E, Zuzuarregui J L, Alamá P, Gamiz P, Crespo J, Bosch E and Pellicer A 2013 The follicular hormonal profile in low-responder patients undergoing unstimulated cycles: Is it hypoandrogenic? Hum. Reprod. 28 224–9
[12] Gleicher N, Weghofer A and Barad D H 2011 The role of androgens in follicle maturation and ovulation induction: friend or foe of infertility treatment? Reprod. Biol. Endocrinol. 9 116
[13] Prizant H, Gleicher N and Sen A 2014 Androgen actions in the ovary: balance is key J. Endocrinol. 222 R141–51.