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Evaluation of Ovarian Reserve in Assisted Reproductive Facilities; Biochemical and Other Alternatives: Pros and Cons-A Review Article

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Abstract

The precarious rate of infertility has become a global issue. The social and psychological stresses attached to the problem of not having a fruit of womb have necessitated the establishment of assisted reproductive facilities within and outside Nigeria. Most of these Centers are privately owned while very few of them belonging to government. The need for the knowledge or idea of ovarian reserve in female clients of fertility centers is very crucial as it will not only tell the suitability of the client for the procedure but also assist in determining the drugs required for stimulation and the protocol required in a particular client. Various methods are being used in evaluation of ovarian reserve in assisted reproductive facilities some of which are biochemical, ultrasonographic, histopathologic and combined in nature and form. This review article is on evaluation of ovarian reserve in assisted reproductive facilities; biochemical and other alternatives as well as their pros and cons. It was essentially based on literatures and research works written in English.

Keywords: Evaluation, Ovarian Reserve, Assisted Reproductive Facilities.

Introduction

The World Health Organization defined infertility as “a disease of the reproductive system characterized by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse (and there is no other reason, such as

breastfeeding or postpartum amenorrhoea (WHO, 2013). Primary infertility is infertility in a couple who have never had a child while secondary infertility is failure to conceive following a previous pregnancy. Infertility may be caused by infection in the man or woman, but often there is no obvious underlying cause. Various other definitions however abound with little or minimal differences. For example, one definition of infertility that is frequently used in the United States by reproductive endocrinologists, doctors who specialize in infertility, to consider a couple eligible for treatment is:

- A woman under 35 years of age who has not conceived after 12 months of contraceptive-free intercourse. Twelve months is the lower reference limit for Time to Pregnancy (TTP) by the World Health Organization (NICE, 2013).
- A woman over 35 years who has not conceived after 6 months of contraceptive-free sexual intercourse.

These time intervals would seem to be reversed; this is an area where public policy trumps science. The idea is that for women beyond age 35, every month counts and if made to wait another 6 months to prove the necessity of medical intervention, the problem could become worse. The corollary to this is that, by definition, failure to conceive in women

under 35 isn't regarded with the same urgency as it is in those over 35.

The precarious rate of infertility has become a global issue. The consequences of infertility are manifold and can include societal repercussions and personal suffering. Advances in assisted reproductive technologies, such as In-Vitro-Fertilization (IVF), can offer hope to many couples where treatment is available, although barriers exist in terms of medical coverage and affordability. The medicalization of infertility has unwittingly led to a disregard for the emotional responses that couples experience, which include distress, loss of control, stigmatization, and a disruption in the developmental trajectory of adulthood (Barratt and Cooke, 1993).

Infertility may have profound psychological effects. Partners may become more anxious to conceive, increasing sexual dysfunction (Edelmann *et al.*, 1994). Marital discord often develops in infertile couples, especially when they are under pressure to make medical decisions.

The social and psychological stresses attached to the problem of not having fruit of womb have necessitated the establishment of assisted reproductive facilities within and outside Nigeria. Most of these centers are privately owned while very few of them belongs to government.

The need for the knowledge or idea of ovarian reserve in prospective female clients of fertility centers is very crucial as it will not only tell the suitability of the client for the procedure but also assist in determining the drugs required for stimulation and the protocol required in a particular client.

Various methods are being used in evaluation of ovarian reserve in assisted reproductive facilities some of which are biochemical, ultrasonographic, histopathological and even combined in nature and form. This review article is on evaluation of ovarian reserve in assisted reproductive facilities (biochemical and other alternatives) as well as their pros and cons. It is essentially based on literatures

and research works written in English and it will be discussed under the following sub-captions:

Assisted Reproductive Technology

Assisted Reproductive Technology (ART) is the use of reproductive technology to treat infertility. As at today the only application of reproductive technology to increase reproduction include *in vitro* fertilization and its possible expansions.

Ovarian reserve

This is a term that is used to determine the capacity of the ovary to provide egg cells that are capable of fertilization resulting in a healthy and successful pregnancy. With advanced maternal age the number of egg cell that can be successfully recruited for a possible pregnancy declines, constituting a major factor in the inverse correlation between age and female fertility.

The concept of “ovarian reserve” defines a woman’s reproductive potential as a function of the number and quality of her remaining oocytes (Schmidt *et al.*, 2005). The general purpose of ovarian reserve testing is to assess the quality and quantity of the remaining oocytes in an attempt to predict reproductive potential. The ideal screening test should be reproducible with low intercycle and intracycle variability and demonstrate high specificity to minimize the risk of a false-positive determination of decreased ovarian reserve in a woman with normal ovarian reserve (Schmidt *et al.*, 2005).

The main goal of ovarian reserve testing is to identify those individuals who are at risk of decreased or diminished ovarian reserve, commonly known as DOR. Although ovarian reserve testing cannot predict the end of one’s reproductive years, results outside the range expected for a patient’s age can encourage the individual to pursue more aggressive treatment options to achieve pregnancy.

The ovary is generally thought of as an egg bank from which the woman draws during her reproductive life. The human ovary contains a population of primordial follicles. At 18–22 weeks post-conception, the female ovary contains its peak



number of follicles (about 300,000 in the average case, but individual peak populations range from 35,000 to 2.5 million) (American Society for Reproductive Medicine, 2012). The size of the initial ovarian reserve is strongly influenced by genetics (*American College of Obstetricians and Gynecologists, 2014*). Also, elevated androgen levels during prenatal development have an adverse effect on the early establishment of the ovarian reserve (*American College of Obstetricians and Gynecologists, 2014*). While there is no known method for assessing the ovarian reserve of individual women (Schmidt *et al.*, 2005), indirect determination of ovarian reserve is important in the treatment of infertility (Te Velde and Pearson, 2002).

Indications for Ovarian Reserve Assessment/Risk Factors for Diminished Ovarian Reserve

- i. Advanced reproductive age (older than 35 years)
- ii. Family history of early menopause
- iii. Genetic conditions (45 X Mosaicism)
- iv. *FMRI* (Fragile X) premutation carrier
- v. Conditions that can cause ovarian injury (endometriosis and pelvic infection)
- vi. Previous ovarian surgery (for endometriomas)
- vii. Oophorectomy
- viii. History of cancer treated with gonadotoxic therapy or pelvic irradiation
- ix. History of medical conditions treated with gonadotoxic therapies
- x. Smoking (American College of Obstetricians and Gynecologists, 2014; Gurtcheff *et al.*, 2011 and Schmidt *et al.*, 2005; Faddy *et al.*, 1992).

Tools for Ovarian Reserve Assessment

These has been classified as follows:

- A. Biochemical markers
- B. Ultrasonographic methods
- C. Histopathological method and
- D. Combined tests method.

A. Biochemical Markers of Ovarian Reserve

i. Basal Follicle-Stimulating Hormone

Follicle-stimulating hormone is released by the pituitary gland in response to gonadotropin-releasing hormone from the hypothalamus and is also subject

to central negative feedback from estradiol and inhibin B. With normal ovarian function, a developing cohort of follicles secretes estradiol and inhibin B, which suppress FSH, keeping it in the normal range. In the setting of a smaller follicular cohort and decreased estradiol and inhibin B levels, an increase in pituitary FSH secretion occurs, which can be identified as an elevated early follicular phase FSH level. This higher FSH level stimulates rapid ovarian follicular growth, which results in higher estradiol levels as well as a shorter follicular phase and reproductive cycle.

Women who are 35 years or older who have attempted to get pregnant unsuccessfully for 6 months should undergo testing for ovarian reserve. The most commonly used test to assess this ovarian reserve is the *day 3 FSH test* (Ferraretti *et al.*, 2011). This blood test determines the level of FSH on cycle day 3. Cycle day 3 is chosen because at this time the estrogen level is expected to be low, a critical feature, as FSH levels are subject to a negative feedback. Thus, any determination of FSH needs to include the corresponding estradiol level to indicate that the FSH level was drawn, when the estrogen level was low. In a patient with infrequent menstruation, an FSH level and estrogen level could be measured at random and is valid if the estrogen level is low. Generally FSH levels are expected to be below 10 MIU/ml in women with reproductive potential (levels of 10-15 MIU/ml are considered borderline), however the exact numbers returned will depend on the type of assay used in a particular laboratory.

With advancing reproductive age, basal serum FSH concentrations increase on days 2–4 of the menstrual cycle. However, because of the inherent variability of each reproductive cycle, the basal FSH level can vary, so a single FSH value has limited reliability (Nelson, 2013). Moreover, there is variability among different FSH assays that further complicates the interpretation of a result. Although basal FSH commonly is used to assess ovarian reserve, and high values (greater than 10–20 international units/L) are associated with diminished ovarian reserve and poor response to ovarian stimulation, the test is not predictive of failure to conceive (Kwee *et*

al., 2004). If FSH values are consistently elevated, a poor reproductive prognosis is likely; in contrast, a single elevated FSH value in women younger than 40 years predicts a lower oocyte yield during IVF but does not affect the rate of pregnancy (Hendriks *et al.*, 2004). It has advantage of widespread use but low sensitivity and limited reliability.

ii. Basal Estradiol

Estradiol is released from the ovary during follicular development. The estradiol level is usually low (less than 50 pg/mL) on days 2–4 of the menstrual cycle but demonstrates some cycle-to-cycle variability. However, an elevated value (greater than 60–80 pg/mL) in the early follicular phase can indicate reproductive aging and hastened oocyte development. Through central negative feedback, a high estradiol level can suppress an elevated FSH concentration into the normal range, so the value of obtaining an estradiol level is that it allows the correct interpretation of a normal basal FSH level. Basal estradiol has low predictive accuracy for poor ovarian response and failure to conceive; therefore, this test should not be used in isolation to assess ovarian reserve (Roberts *et al.*, 2005).

iii. Anti-Müllerian Hormone

Anti-müllerian hormone is a glycoprotein hormone that is produced by the granulosa cells of primary, preantral, and antral follicles 2–6 mm in diameter; thus, it reflects the size of the primordial oocyte pool (Ferraretti *et al.*, 2011). Because early follicles secrete anti-müllerian hormone in a gonadotropin-independent state, the anti-müllerian hormone concentration is fairly stable within and between menstrual cycles (Broekmans *et al.*, 2006). As the number of ovarian follicles decreases with age, a concomitant decrease in anti-müllerian hormone levels occurs, which reflects this age-related oocyte depletion (Tsepelidis *et al.*, 2007). Although an undetectable anti-müllerian hormone level suggests diminished ovarian reserve and can identify individuals at risk of poor ovarian response to stimulation, undetectable and low anti-müllerian hormone levels (0.2–0.7 ng/mL DSL ELISA) are not predictive of failure to conceive (Bentzen *et al.*, 2013). Furthermore, anti-müllerian hormone levels may allow treatment to be tailored to each

individual. In vitro fertilization protocols take into consideration that lower anti-müllerian hormone levels are associated with reduced ovarian response to stimulation, and high levels raise concern for a brisk ovarian response to stimulation (Bentzen *et al.*, 2013). Although the level of anti-müllerian hormone is a good predictor of oocyte quantity, it may not provide information about egg quality. Thus, young women with low anti-müllerian hormone levels may have a reduced number of oocytes but normal, age-appropriate oocyte quality (Nelson *et al.*, 2007).

One limitation of anti-müllerian hormone level testing is the variability of results between the available assays and the inability to compare anti-müllerian hormone levels when different assays are used. Therefore, in clinical practice, individual anti-müllerian hormone level test results must be interpreted based on the reference limit/interval of the assay used, and standard cut-off points may differ based on the assay (Toner *et al.*, 2013). Anti-müllerian hormone level testing is a useful screening test in women at high risk of diminished ovarian reserve and in women undergoing IVF but has limited benefits in someone at low risk of diminished ovarian reserve (Toner *et al.*, 2013).

With further research, anti-müllerian hormone level testing may become increasingly valuable in assessing ovarian reserve for young women with cancer (American Society for Reproductive Medicine, 2012). Measuring anti-müllerian hormone levels before and after chemotherapy allows detection of differences in ovarian toxicity among chemotherapy regimens and may help evaluate long-term ovarian function (Peigne and Decanter, 2014 and American Society for Reproductive Medicine, 2012). Further research on anti-müllerian hormone may enable assessment of ovarian reserve before and after ovarian surgery and for women at high risk of primary ovarian insufficiency; this research may provide an accurate method of predicting the reproductive lifespan and the timing of menopause (Broekmans *et al.*, 2006).

iv. Inhibin B

Inhibin B is a glycoprotein hormone that is secreted primarily by pre-antral and antral follicles. The



serum concentration of inhibin B decreases with the age-related decrease in the number of oocytes. Inhibin B has central negative feedback that controls FSH secretion; therefore, a decrease in inhibin B levels leads to increased pituitary FSH secretion and higher early follicular FSH levels. However, there is significant variability in inhibin B levels between menstrual cycles. This marker does not reliably predict a poor response to ovarian stimulation and, thus, is not a recommended test (Nielsen *et al.*, 2013). It is very sensitive with little reliability.

v. **Clomiphene Citrate Challenge Test**

The clomiphene citrate challenge test (CCCT) is performed by measuring serum FSH on cycle day 3, administering 100-mg clomiphene citrate daily on cycle days 5–9, and again measuring serum FSH on cycle day 10. In women with a reduced number of ovarian follicles, lower estradiol and inhibin B production leads to less central negative feedback of FSH secretion and an elevated FSH level after clomiphene stimulation. Therefore, an elevated FSH level on day 10 of the clomiphene citrate challenge test is suggestive of diminished ovarian reserve. However, there is cycle-to-cycle variability in ovarian biomarkers (estradiol, inhibin B, and FSH) during the clomiphene citrate challenge test, which limits the reliability of this provocative test. The stimulated FSH level on cycle day 10 of the clomiphene citrate challenge test is predictive of poor ovarian response but is not predictive of failure to conceive (Jayaprakasan *et al.*, 2010). Compared with the basal FSH level and the antral follicle count, the cycle-day-10 FSH level does not improve the prediction for poor ovarian response (Jayaprakasan *et al.*, 2010). Therefore, the use of this test to assess ovarian reserve is not recommended (Jayaprakasan *et al.*, 2010). It has higher sensitivity than basal FSH but less reliable.

vi. **Home Fertility Tests**

Available home fertility tests use a urine sample to assess the FSH level on cycle day 3. These tests are marketed directly to consumers and claim high accuracy in determining a woman's ability to conceive. The limitations of these tests include misinterpretation of instructions and results and the unavailability of a medical professional to interpret

and explain the results (Toner *et al.*, 2013). Although these tests are used commonly by women at low risk of diminished ovarian reserve, the results may provide false reassurance or raise unnecessary concern.

B. Ultrasonographic Method

i. Antral Follicle Count

The antral follicle count records the number of visible ovarian follicles (2–10 mm mean diameter) that are observed during transvaginal ultrasonography in the early follicular phase (cycle days 2–5). The number of antral follicles correlates with the quantity of remaining follicles and with the ovarian response during controlled ovarian stimulation, and good intercycle and inter observer reliability has been demonstrated (Nielsen *et al.*, 2013 and Tsepelidis *et al.*, 2007). A low antral follicle count is considered 3–6 total antral follicles and is associated with poor response to ovarian stimulation during IVF, but it does not reliably predict failure to conceive; in a meta-analysis, a low antral follicle count was a mean of 5.2 (2.11 standard deviation) total antral follicles (Tsepelidis *et al.*, 2007). There are limited data on the predictive capacity of antral follicle count for IVF patients, so it should not be the sole criterion used to plan treatment (Toner *et al.*, 2013). When antral follicle count was compared with age, basal FSH, basal estradiol, antimüllerian hormone, inhibin B, and ovarian volume, antral follicle count and antimüllerian hormone were the most significant predictors of poor response to ovarian stimulation but were not predictive of failure to conceive (Nielsen *et al.*, 2013).

ii. Ovarian Volume

The calculation of ovarian volume requires ovarian measurements in three planes and the use of the formula for the volume of an ellipsoid: $D1 \times D2 \times D3 \times 0.52$. Some ultrasound software may calculate this value automatically. Mean ovarian volume, the average volume calculated for both ovaries from the same individual, is the value used to assess ovarian reserve. With age, changes in ovarian volume are concordant with the age-related decrease in ovarian follicles. Although ovarian volume correlates with ovarian response to stimulation, it does not predict

failure to conceive (Tsepelidis *et al.*, 2007). When screening for diminished ovarian reserve with imaging, ovarian volume has limited value

C. Histopathological Method

Ovarian Biopsy

Ovarian reserve depends on the number of primordial follicles in the ovarian cortex. It was suggested that determining the follicular density directly by obtaining ovarian biopsy might be more accurate than current indirect biochemical and ultrasonic tests, especially for women in the later stage of their reproductive life.

The pool of primordial follicles in the ovary or 'ovarian reserve' is a major factor in human fertility potential. The ageing ovary is characterized by reduction of the number of primordial follicles, and this loss accelerates in the late 30s and precedes the menopause by 10±12 years (Hendriks *et al.*, 2006). The definite pool of primordial follicles is relevant in two major aspects: (i) the fertility potential of women especially in the later part of their reproductive life; and (ii) in women suffering from malignant disease who are going through ovarian biopsies collection as a potential measure to preserve their fertility capacity. For these women, not only the presence of follicles but their location and whether they exist in the preserved biopsies are important. Ovarian biopsy has been suggested to be considered as part of infertility evaluation (Richardson *et al* 1987).

In recent years, intensive research has been conducted to try to predict the ovarian reserve, in particular before embarking on fertility treatment. Randomized or 'blind' single biopsy is adequate if follicles are evenly spread in the ovarian cortex (in any case, they are not deeper than 2 mm from the surface (Amil, 2004). The distribution of follicles was extremely uneven in ovarian tissue. A large variation in follicle numbers was observed in ovarian tissue samples from patient to patient. Furthermore, even though some tissue samples were originally obtained from the same patient, the number of follicles counted in one sample of ovarian tissue did not match the number found in another tissue sample (Kohl *et al.*, 2000 and Amil, 2004). compared samples of contralateral ovaries from

women and multiple biopsies from different sites in another two patients, all of them from women having diagnostic laparoscopy for infertility. They found large variation between the ovaries and between the sites of the biopsies compared with antral follicle count for detection of diminished ovarian reserve (Qu *et al.*, 2000).

D. Combined Ovarian Reserve Tests

A variety of biochemical and imaging techniques can be used to evaluate ovarian reserve. Because no single assessment of ovarian reserve has 100% sensitivity and specificity, tests often are combined in an attempt to improve the prediction of poor outcomes. Anti-müllerian hormone and antral follicle count are the most accurate predictors, but combinations of a few tests are only slightly better than a single test. Because of the heterogeneity of the tests and cut-off points used in research studies, models of combined ovarian reserve tests do not significantly improve the ability to predict poor reproductive outcomes over single ovarian reserve tests (Toner *et al.*, 2013). Furthermore, the use of multiple ovarian reserve tests may complicate the understanding of an individual's ovarian reserve and increase the expense of screening. Further research is needed to determine an optimal combination of tests. An improved understanding of the genetic basis of ovarian aging may enable the development of a panel of genetic tests for routine screening for ovarian aging.

Suggestions, Recommendations and Conclusions

Based on the available data and expert opinion, the American College of Obstetricians and Gynecologists offers the following suggestions, recommendations and conclusions:

- Ovarian reserve testing should be performed for women older than 35 years who have not conceived after 6 months of attempting pregnancy and women at higher risk of diminished ovarian reserve, such as those with a history of cancer treated with gonado-toxic



therapy, pelvic irradiation, or both; those with medical conditions who were treated with gonadotoxic therapies; or those who had ovarian surgery for endometriomas.

- For general Obstetrician–Gynecologists, the most appropriate ovarian reserve screening tests to use in practice are basal follicle-stimulating hormone (FSH) plus estradiol levels or antimüllerian hormone (commonly known as AMH) levels. An antral follicle count, commonly known as an AFC, also may be useful if there is an indication to perform transvaginal ultrasonography.
- It is important to recognize that a poor result from ovarian reserve testing does not signify an absolute inability to conceive and should not be the sole criteria considered to limit or deny access to infertility treatment.
- The best surrogate marker for oocyte quality is age.
- Female age and ovarian reserve test results are useful for discussing prognosis and recommending a treatment plan. Younger women with diminished ovarian reserve demonstrate reduced oocyte numbers but may have normal oocyte quality, whereas older women with normal ovarian reserve may have a good number of oocytes but an age-appropriate decrease in oocyte quality.
- When test results suggest decreased or diminished ovarian reserve, it is reasonable to counsel the woman that her window of opportunity to conceive may be shorter than anticipated, and attempting to conceive sooner rather than later is encouraged.
- Compared with women of similar age, women with diminished ovarian reserve commonly have regular menses but a reduced quantity of ovarian follicles and, thus, may have a limited response to ovarian stimulation with fertility medications and reduced fecundity (probability

of achieving a live birth in a single reproductive cycle).

Conclusively, the primary goal of ovarian reserve testing is to identify women at risk of decreased or diminished ovarian reserve with a secondary goal of individualizing treatment strategies for each woman. Although these tests may predict ovarian response to infertility treatment, they do not reliably predict failure to conceive (Roberts *et al.*, 2005 and Schmidt *et al.*, 2005).

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