



**COMPARISON BETWEEN THE FERTILITY RATE AMONG SELECTED GROUP OF  
URBAN AND RURAL SUDANESE MALES APPLYING HYALURONAN BINDING  
ASSAY METHOD**

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Article Received on 15/01/2018

Article Revised on 04/02/2018

Article Accepted on 25/02/2018

**ABSTRACT**

**Background:** Sperm hyaluronan binding may be an indicator of which sperm are most likely to produce a viable pregnancy. For example, mature, hyaluronan-binding sperm are essentially free of cytoplasmic inclusions. This is of great value since cytoplasmic inclusions are extremely difficult to see when selecting sperm to inject into eggs in an in vitro fertilization cycle. **Rationale:** The data concerning fertility rate of rural and urban Sudanese males' populations is extremely rare. **Objectives:** To know the fertility rate among rural in comparison to urban Sudanese males. **Method:** Men who are preparing to do in vitro fertilization with ICSI will be asked to collect a semen specimen in the same manner that they would for a conventional semen analysis. The semen is mixed with some media and placed on a special slide that has been coated with hyaluronan. Mature sperm will bind the hyaluronan (bound). These sperm will appear to have their heads stuck but with tails that show vigorous tail motion. Immature sperm will move freely (unbound). **Result:** Average of HBA of urban: 76.4% (normal) Average of HBA rural: 64.25% (lower than normal) **Discussion:** Fertility rate among urban males was higher than that of rural males which is differ from previous studies mentioned above, that may be attributed to environmental, occupational, nutritional and social factors. **Conclusion:** We conclude that the fertility rate among rural participants was lower than that of urban participants. **Recommendations:** Further studies must be done with large sample size and more fertility assessment methods. **Acknowledgement:** To all participants in the study for their collaboration and cooperation with the research team.

**KEYWORDS:** Fertility rate, urban, rural, hyaluronan binding assay.

**INTRODUCTION**

The most important part of the management of male infertility is a correct diagnosis. The semen analysis is widely performed as a major test of male fertility potential, by assessing sperm count, motility and morphology of the spermatozoa. It is clear that these parameters are not sufficient alone to interpret the fertility status of an ejaculate, unless significantly abnormal. Sperm function may not be predicted by semen analysis, as the fertilization process involves a large number of biochemical events not measured by these parameters. Thus, semen analysis is limited in its inability to assess the fertilizing potential of the sample. Nearly one third of male factor infertility etiologies remain unexplained and are considered idiopathic. Additional tests need to be used to indicate the functional activity of spermatozoa. The sperm penetration assay (SPA) is one such test that provides additional information for sperm fertilizing ability, using zona free hamster oocytes. Unfortunately, the SPA is costly,

technically challenging, time consuming and is not readily performed in many infertility clinics. We chose to examine a less costly, technically easier alternative for assessing sperm function that could serve as a useful screening tool to aid in the decision making process to determine which appropriate reproductive techniques should be used.<sup>[1]</sup>

The HBA Assay is a diagnostic tool with dual Hyaluronan coated chambers for sperm sample evaluation.

The Sperm-Hyaluronan Binding Assay is designed to provide a qualitative assessment of sperm quality, maturity, and fertilizing potential.

It allows you to distinguish between mature sperm that express Hyaluronan receptors and those that do not. Assessing the proportion of sperm with Hyaluronan

receptors can then be used to decide which treatment is best for your patient.<sup>[2]</sup>

Hyaluronan is a type of sugar known as a high molecular weight glycosaminoglycan. Hyaluronan is found in many parts of the body. Most importantly, hyaluronan is a key component of the group of cells that surround the egg (the cumulus oophorous). During the final stages of sperm maturation, the sperm develop the ability to bind (attach) to hyaluronan. Research has shown that hyaluronan binding is an important indicator of sperm health and maturity. It appears that the attachment of sperm to the hyaluronan surrounding the egg serves as a natural selection mechanism for mature sperm during normal conception.

#### **Markers of sperm health and maturity**

Beyond the parameters that we look at in a normal semen analysis, there are other markers of sperm health and maturity.

#### **Cytoplasmic inclusions**

During the final stages of sperm maturation, a normal sperm must get rid of excess cytoplasm. Defects in the normal development of sperm may result in excess cytoplasm being retained near the sperm head. This is known as cytoplasmic retention or inclusions. These are visible with a high powered microscope.

#### **Sperm creatine phosphokinase (CK)**

Elevated levels have been shown in a number of studies to be associated with defective sperm function and lower pregnancy rates.

#### **HspA2 Chaperone Protein**

This protein is found in higher levels in normal sperm. Sperm with low levels are more likely to have chromosome abnormalities and DNA fragmentation.

#### **Sperm Hyaluronan Binding**

Sperm hyaluronan binding may be an indicator of which sperm are most likely to produce a viable pregnancy. For example, mature, hyaluronan-binding sperm are essentially free of cytoplasmic inclusions. This is of great value since cytoplasmic inclusions are extremely difficult to see when selecting sperm to inject into eggs in an in vitro fertilization cycle.

#### **The Hyaluronan Binding Assay work**

Men who are preparing to do in vitro fertilization with ICSI will be asked to collect a semen specimen in the same manner that they would for a conventional semen analysis. The semen is mixed with some media and placed on a special slide that has been coated with hyaluronan. Mature sperm will bind the hyaluronan (bound). These sperm will appear to have their heads stuck but with tails that show vigorous tail motion. Immature sperm will move freely (unbound).

We then calculate the percentage of bound sperm. This is the HBA score. A normal HBA score is greater than 70%. An abnormal HBA score is less than 70%.

For all men with an abnormal HBA score, when they have ICSI performed in the lab, the embryologists will use media containing hyaluronan to select healthy sperm for injection.<sup>[3]</sup>

Approximately 10 to 15% of couples are impacted by infertility. Recently, the pivotal role that lifestyle factors play in the development of infertility has generated a considerable amount of interest. Lifestyle factors are the modifiable habits and ways of life that can greatly influence overall health and well-being, including fertility. Many lifestyle factors such as the age at which to start a family, nutrition, weight, exercise, psychological stress, environmental and occupational exposures, and others can have substantial effects on fertility; lifestyle factors such as cigarette smoking, illicit drug use, and alcohol and caffeine consumption can negatively influence fertility. It has been estimated that 7.4% of women and their husbands in the United States are infertile and that the number of infertile people in the world may be as high as 15%, particularly in industrialized nations.<sup>[4]</sup>

#### **Literature review**

Study done by Kulu H in Finland showed that; fertility levels are the highest in small towns and rural areas and the lowest in the capital cities.<sup>[5]</sup>

Study done by Li S and Wang W in China showed that, the proportion of urban population with similar rates of fertility with rural areas would have produced 28.77%, but census figures indicate urbanization to be 26.23%. The imbalance in urban and rural fertility rates has increased urbanization by 2.54%.<sup>[6]</sup>

#### **Rationale**

The data concerning fertility rate of rural and urban Sudanese males' populations is extremely rare.

#### **Objectives**

To know the fertility rate among rural in comparison to urban Sudanese males.

#### **MATERIAL AND METHODS**

**Study design:** descriptive study.

**Study period:** January to December 2017.

**Sample size:** 40, equally divided into 20 urban Sudanese males and 20 rural Sudanese males.

**Study population:** Sudanese adults' males resident in towns or villages.

#### **Selection criteria**

##### **Inclusion criteria**

- Sudanese
- Adult
- Male

- Married from one year or more
- Live permanently in village or town.

**Exclusion criteria**

- Not Sudanese
- Child
- Female
- Single

**Ethical consideration**

All participants were informed about the objectives of the study and their consents were obtained before sampling.

**Method**

**Specimen:** seminal fluid.

**Technique**

Commercial HBA kits were purchased from Biocoat, and the HBA test was performed following the manufacturer's instructions. Briefly, 10 µl of semen (well mixed) was added to the centre of the HBA chamber and the Cell-Vu grid cover slip was put on without entrapping air bubbles. The cover slip provided a grid of 100 squares (each 0.1 mm × 0.1 mm) within a viewing circle. After incubation of the slide for 15 min, the unbound motile sperm and the bound motile sperm were counted in the same grid squares. For the HBA test, 400 motile sperm were counted. The percentage of hyaluronan-binding sperm was calculated using the bound motile sperm divided by the sum of bound and unbound motile sperm counted in the same squares and then multiplied by 100.

**RESULTS**

Average of HBA of urban: 76.4%

Average of HBA rural: 64.25%

**DISCUSSION**

Fertility rate among urban males was higher than that of rural males which is differ from previous studies mentioned above, that may be attributed to environmental, occupational, nutritional and social factors.

**CONCLUSION**

We conclude that the fertility rate among rural participants was lower than that of urban participants.

**Recommendations**

Further studies must be done with large sample size and more fertility assessment methods

**ACKNOWLEDGEMENT**

To all participants in the study for their collaboration and cooperation with the research team.

**REFERENCES**

1. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2868308/>
2. <http://www.origio.com/products/hba-assay/>
3. <https://www.ivf1.com/hyaluronan-binding-assay/>
4. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3717046/>.
5. <http://www.tandfonline.com/doi/abs/10.1080/00343404.2011.581276?src=recsys&journalCode=cres20>
6. <https://www.ncbi.nlm.nih.gov/pubmed/12288643>