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Does Gonadotropin Dosing during Ovarian Stimulation Influence the Quality of the Retrieved Ooctes

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Abstract: ***Introduction: Ovarian stimulation during assisted reproductive technology (ART) cycles requires exogenous gonadotropins to allow for the retrieval of multiple oocytes which can be used for ART. The total gonadotropin dose used for ovarian stimulation is dependent on patient characteristics like age, BMI, ovarian reserve and responsiveness to follicular stimulating harmone (FSH). Hence, patients who are poor responders tend to receive higher doses. The success of in vitro fertilization (IVF) depends on both the number and the quality of retrieved oocytes. Aim: To analyse the association between the gonadotropin dose used for controlled ovarian stimulation and the quality of oocytes retrieved among the subfertile women. Method: In this retrospective study conducted in the department of Institute of Reproductive Medicine and women's health, Madras Medical Mission hospital all subfertile women and oocytes donor between age group 21 – 40 years who underwent In Vitro Fertilisation (IVF) treatment oocyte retrieval from 2019 to 2021 were included. Women with previously known poor quality oocytes, aged above 41 years were excluded. The study population was divided into three groups based on the gonadotropin dosage used for stimulation. Total gonadotropin dose refers to the total dose of FSH and human menopausal gonadotropin (hMG) preparations. Group 1: ≤ 3000 IU , Group 2: 3001 – 4000 IU, Group 3: ≥ 4001 IU. Quality of oocytes assessed using 6 parameters creating a Total oocyte score (TOS) ranging between +6 to - 6. Results: Gonadotropins dose used for stimulation ranged between 1425 IU - 6300 IU. The duration of stimulation ranged between 8 - 13 days with an average of 10 days. There was statistically significant correlation between the duration of stimulation, gonadotropin dose and the quality of oocytes. Mean oocyte score in the group which received doses between 3001 - 3999 IU was 2.7 ± 1.1, which was higher than the score observed in groups which received less or more gonadotropins (Chart 10). At doses above 4000 IU the mean score was found to be less than 2 (i.e. 1.8 ± 1.4) Conclusion: Recruiting few oocytes that have the potential to fertilize and develop into a competent embryo with a high implantation capacity is the primary requirement, rather than striving for a more number of oocytes. Even in predicted poor responders – starting with standard high dose of gonadotropins due to fear of cycle cancellation should be reconsidered. It is the quality which matters not the quantity***

Keywords: Gonadotropin dose, ovarian stimulation, oocyte quality, total oocyte score, mean oocyte score

1. Introduction

Subfertility is a state which has potential impact on the quality of life of both women and men. Reproduction is considered to be one of the key elements of life and failing to create offspring leads to mental stress, frustration and physical health problems, long treatment schedules. The advent of IVF has paved way for problems such as tubal disease, severe male factor, anovulation states, and unexplained infertility.

Ovarian stimulation during assisted reproductive technology (ART) cycles utilizes exogenous gonadotropins to allow for the retrieval of multiple oocytes which can be used for ART. The total gonadotropin dose used for ovarian stimulation is dependent on patient characteristics like age, BMI, ovarian reserve and responsiveness to FSH. Hence, patients who are poor responders tend to receive higher doses.

The success of *in vitro* fertilization (IVF) depends on both the number and the quality of retrieved oocytes. The quality of the oocytes that arrive after follicle aspiration is considered to be the most important factor. Good quality oocytes when handled under optimal laboratory conditions develop into good quality embryos which when placed smoothly at the right time in the uterine cavity, create the highest chances for having a baby from the ART cycle.

Will the approach in stimulation and egg retrieval make a difference for the oocyte quality? Many clinicians today follow the idea that more oocytes will lead to a better outcome, especially regarding live birth rates. Such belief strongly refuted by various studies.

Aim

To analyse the association between the gonadotropin dose used for controlled ovarian stimulation and the quality of oocytes retrieved among the subfertile women.

2. Materials and Method

In this retrospective study conducted in the department of Institute of Reproductive Medicine and women's health, Madras Medical Mission hospital all subfertile women and oocytes donor between age group 21 – 40 years who underwent In Vitro Fertilisation (IVF) treatment oocyte retrieval from 2019 to 2021 were included. Women with previously known poor quality oocytes, aged above 41 years were excluded.

The study population was divided into three groups based on the gonadotropin dosage used for stimulation. Total gonadotropin dose refers to the total dose of FSH and human menopausal gonadotropin (hMG) preparations.
Group 1: ≤ 3000 IU
Group 2: 3001 – 4000 IU
Group 3: ≥ 4001 IU

Controlled ovarian stimulation: Subfertile women and oocytes donors are stimulated from the second day of cycle using hMG or recombinant FSH. Standard fixed antagonist protocol using Cetorelix was started from day 5 of stimulation.

Follicular growth monitoring was done by transvaginal ultrasound, from day 4 or day 5 of the gonadotropin administration.

Trigger: Human chorionic gonadotrophins (HCG) alone or dual trigger - HCG and Gonadotrophin releasing harmone (GnRH) agonist (Triptorelin) given when > 3 follicles reach 17 mm size.

Oocyte retrieval is done 35 hours after trigger by transvaginal, transabdominal or transcervical approach using a Cook's double lumen egg collection catheter with an aspiration pressure of 140 - 150mmHg. After 2 hours of incubation, oocytes are denuded from their cumulus complex using the enzyme hyaluronidase following which the morphology and quality of oocytes assessed using 6 parameters

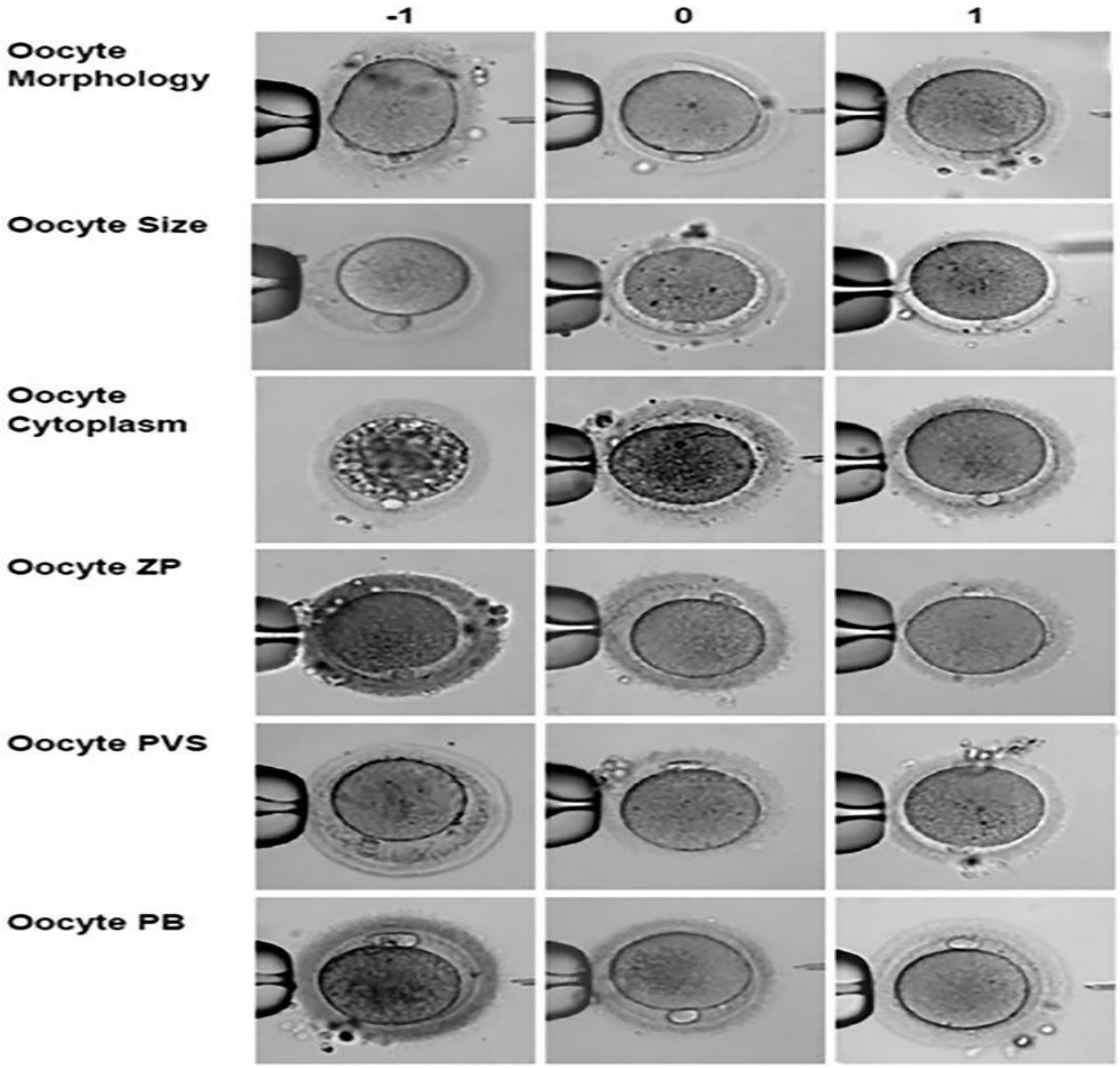
Oocyte Assessment
After 2 hours incubation in culture medium (G MOPS), oocytes are denuded from their cumulus complex by enzymatic and mechanical methods. Using an inverted microscope the oocyte maturity is assesed and classified into three categories.

- Metaphase II (MII) (presence of the first polar body),
- Metaphase I (MI) (absence of the first polar body and germinal vesicle breakdown)
- Germinal vesicle (GV)

Quality of oocytes assessed using 6 parameters creating a Total oocyte score (TOS) ranging between +6 to - 6.

(Source: *Emanuela Lazzaroni - Tealdi, David H. Barad David F. Albertini Yao Yu, Vitaly A. Kushnir, Helena Russell, Yan - Guang Wu, Norbert Gleicher*: Oocyte Scoring Enhances Embryo - Scoring in predicting Pregnancy Chances with IVF Where It Counts Most)

Parameters	+1	0	- 1
Oocyte morphology	Normal oocyte coloration, round shape	Less dark general oocyte coloration and less ovoid shape	dark general oocyte coloration and/or ovoid shape
Oocyte size	> 130 μm and <150 μm	Size did not deviate from normal by more than 10 μm	Size below 120μm or greater 160μm
Ooplasm	Absence of granularity and inclusions	Slightly granular and/or demonstrated only few inclusions	Very granular and/or very vacuolated and/or demonstrated several inclusions
PVS	Normal size PVS with no granules.	Moderately enlarged PVS and/or small PVS and/or a less granular PVS	Abnormally large PVS, an absent PVS or a very granular PVS.
Zona pellucida	Normal zona (> 12 μm and <18 μm)	Did not deviate from normal by more than 2 μm	Very thin or thick (<10μm or >20μm)
Polar body	Round shape and clear borders.	Fair but not excellent	Flat and/or multiple PBs, granular and/or either abnormally small or large PBs



Statistical analysis performed using SPSS software. Baseline characteristics like age, BMI, Anti mullerian harmone, antral follicle count were compared with the oocyte quality using independent t test. Statistical significance will be defined as p value < 0.05. Correlation between the gonadotropin dose and number of oocytes, their quality was analysed using Pearson's 'R' correlation and sig 2 tailed value. Independent t test was used to identify the association between dose of gonadotropins, duration of stimulation and oocyte quality

The primary outcome analysed was the oocyte quality. Oocyte quality was categorised as poor quality, average quality and good quality oocytes. Oocytes with a score between - 6 to - 3 were considered as poor quality ocytes, those between - 2 to 2 were considered average quality and those between +3 to +6 were good quality oocytes. The secondary outcomes include duration of stimulation, number of retrieved oocytes, number of mature oocytes.

3. Results

During the study period 175 patient's case records were analysed. The baseline characteristics of patients including the age, BMI, AMH, AFC, dose of gonadotropins required for stimulation and the duration of stimulation were listed in table 1. The various indications for ovarian stimulation and IVF in the study population was mentioned in the chart 1. Among the various indications oocyte quality was found to be better in male factor and unexplained groups, where > 55 % had a total oocyte score of ≥ + 3.

Table 1: Baseline characteristics of the study population

	BMI (kg/m^2)	Age (years)	AMH (ng/ml)	AFC	Dose of gonadotropin (IU)	Days of stimulation	No of follicles	No of oocytes	M2	AVG TOS
Mean	26.976	30.93	4.100	17.20	3644.80	10.57	11.72	11.83	10.01	2.35
Median	26.000	30.00	3.350	16.00	3600.00	10.00	10.00	11.00	9.00	2.00
Minimum	15.000	23	0.140	3	1425	8	1	1	1	- 2
Maximum	40.300	45	11.400	42	6300	13	34	43	29	5

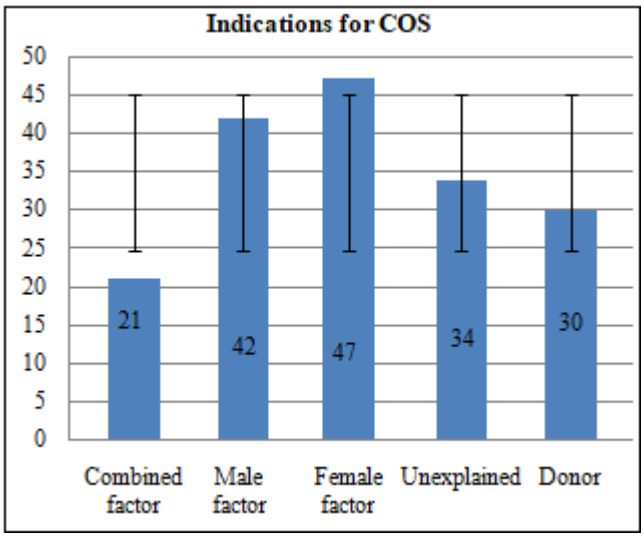


Chart 1: Indications for controlled ovarian stimulation

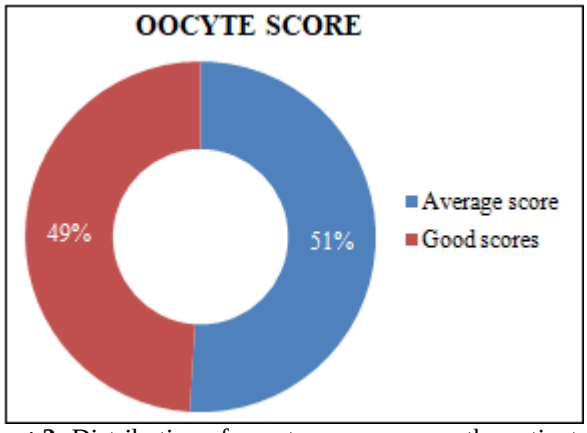


Chart 2: Distribution of oocyte scores among the patients in our study

The number of eggs collected per person 10.01 ± 3.7. Based on the six parameters (morphology, size, cytoplasm, PVS, Zona pellucida, polar body) scores were givena and the quality of oocytes was assessed. The average total oocyte score 2.35± 0.345. In our study group there were no cases with poor oocyte quality.51% had average quality oocytes and 49% had good quality oocytes. (Chart 2)

	Oocyte quality	Mean ±SD	p value
BMI (kg/m^2)	Average (n =89)	27.10 ± 4.39	0.694
	Good (n= 85)	26.83± 4.67	
AGE (years)	Average (n=89)	31.03±4.91	0.754
	Good (n=85)	30.81±4.38	
AMH (ng/ ml)	Average (n=89)	3.83±2.89	0.235
	Good (n = 85)	4.37±2.78	
AFC	Average (n=89)	16.43± 8.47	0.207
	Good (n=85)	18.00±7.89	

Table 2: Baseline characteristics and oocyte quality analysed using independent t test. (statistical significance p value <0.05)

Table 2 demonstrates the relationship between patients characteristics and the oocyte quality. It was found that as the BMI increases the quality of oocytes also compromises. Not surprisingly, increasing age was also associated with decreased quality of oocytes. AMH values above 4ng/ m L yielded good quality oocytes, whereas low AMH values are associated lower quality oocytes. Although the patients characteristics like age, BMI, AMH and AFC were found to have an association with the quality of oocytes, it was statistically significant.

Dose of gonadotropins

Gonadotropins dose used for stimulation ranged between 1425 IU – 6300 IU. In our study 25% received doses ≤3000 IU, 42% received doses between 3001 - 3999 IU and 33% received doses ≥ 4000 IU (Chart 3).

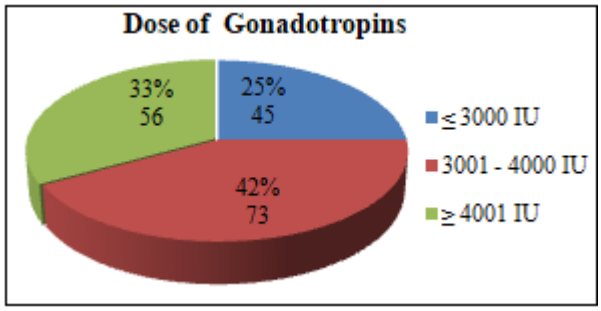


Chart 3: Diagram depicting the distribution of patients based on the dosage used for stimulation

The dose of gonadotropins required for stimulation increased with increasing BMI as depicted in the scatter plot shown in chart 4.

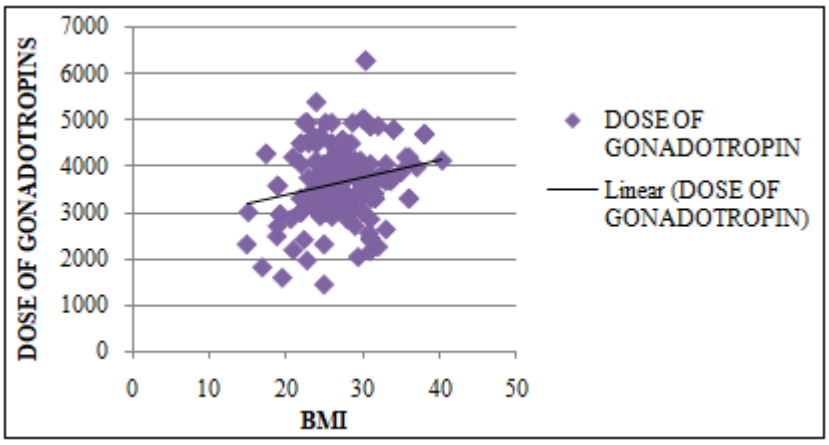


Chart 4: Relationship between BMI and gonadotropin dose requirement

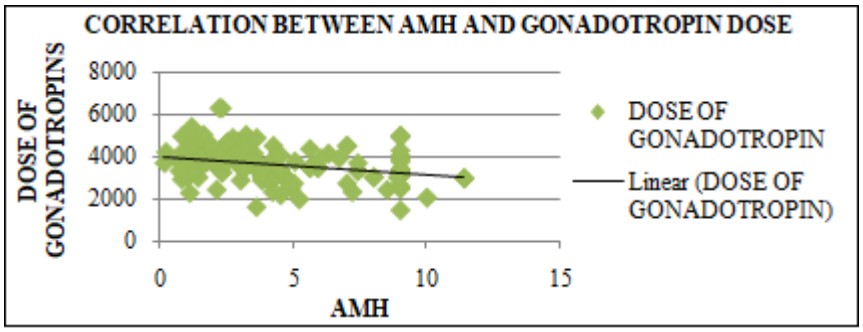


Chart 5: Relationship between AMH and gonadotropin dose requirement

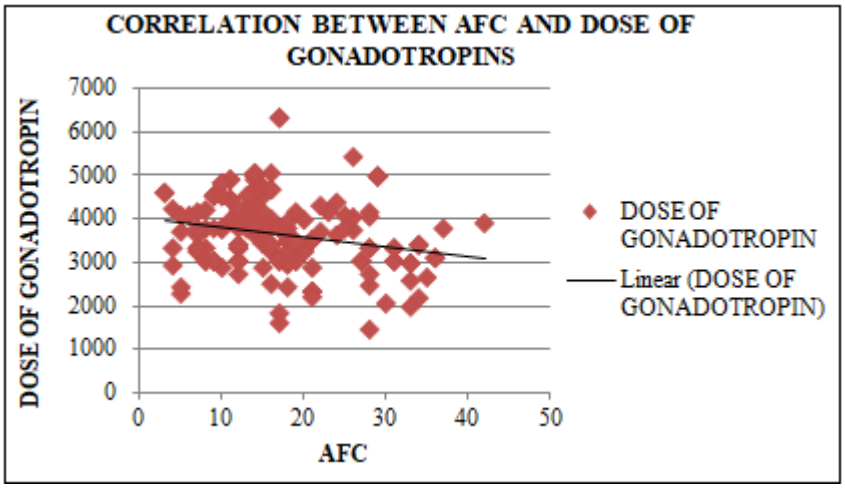


Chart 5, 6: Relationship between AFC and gonadotropin dose requirement

AMH and AFC had a negative correlation with the dosage of gonadotropins, implying that patients with poor ovarian reserve received higher doses for stimulation. (Chart 5, 6)

As the dose of gonadotropins increased, the number of oocytes retrieved and the number of mature oocytes was reduced. This negative correlation between the dose of gonadotropins and the number of oocytes was depicted in the scatter plot shown below (Chart 7, 8)

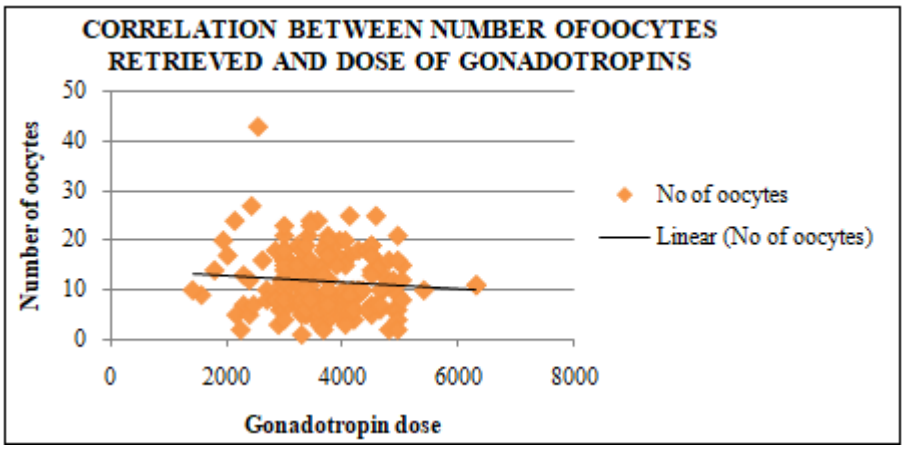


Chart 7: Scatter plot showing negative correlation between number of oocytes retrieved and the dose of gonadotropins

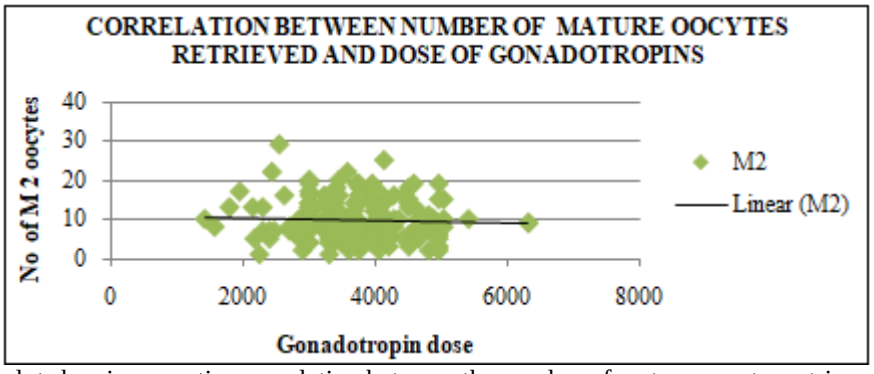


Chart 8: Scatter plot showing negative correlation between the number of mature oocytes retrieved and the dose of gonadotropins used for stimulation.

It was found that there was statistically significant correlation between the gonadotropin dose and the quality of oocytes. Scatter plot of the two variables show a negative correlation between the dose and oocyte quality (chart 9, table 3).

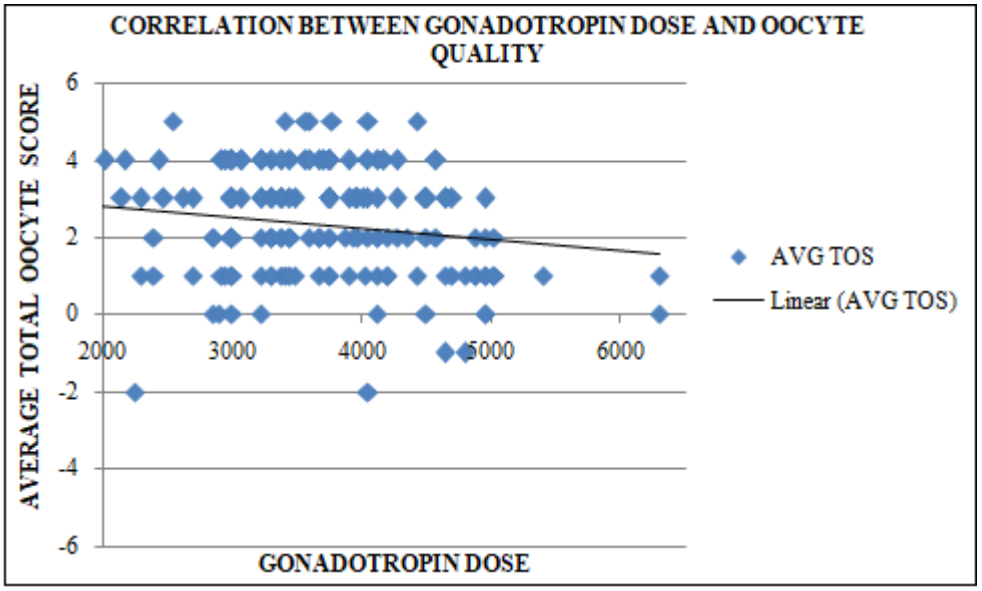


Chart 9: Scatter plot showing correlation between gonadotropin dose and oocyte quality

Table 3: Independent t test showing association between dose of gonadotropins, duration of stimulation and oocyte quality. (Statistical significance is p value <0.05)

	Quality of oocyte	Mean ± SD	p value
DOSE OF GONADOTROPIN	Average (n=89)	3770.35 ± 911.571	**0.038**
	Good (n=85)	3513.35 ± 693.189	
DOSE OF LH	Average (n=89)	2825.22 ± 1296.431	**0.005**
	Good (n=85)	2282.55 ± 1194.339	
DAYS OF STIMULATION	Average (n=89)	10.79 ± 1.266	**0.015**
	Good (n=85)	10.35± 1.055	

Mean oocyte score in the group which received doses between 3001 - 3999 IU was 2.7 ± 1.1, which was higher than the score observed in groups which received less or more gonadotropins (Chart 10). At doses above 4000 IU the mean score was found to be less than 2 (ie 1.8 ± 1.4) and this association was statistically significant. There were no statisctically significant difference in the number of total oocytes and mature oocytes retrieved between the groups. (Table 4)

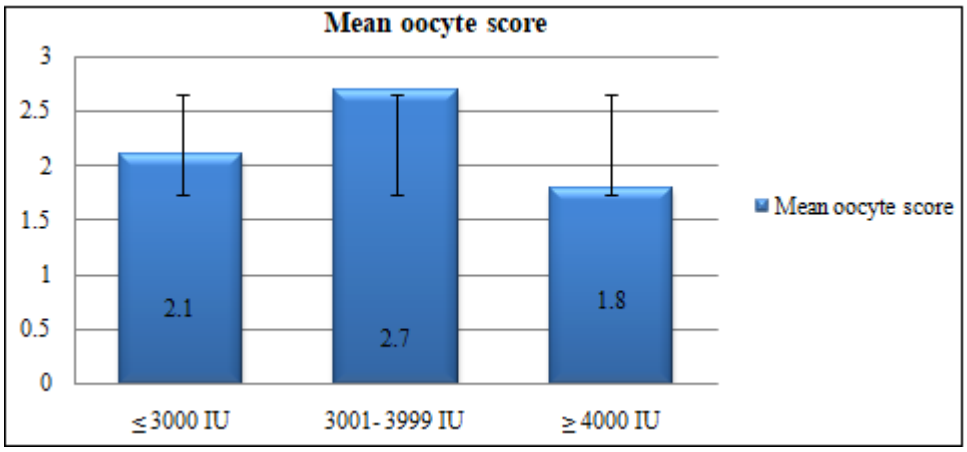


Chart 10: Table showing the mean oocyte scores in the various gonadotropin dosage groups

Table 4: Comparison of the outcomes between the three groups of the study population

	Group 1 ≤ 3000 IU			Group 2 3001 – 3999 IU			Group 3 ≥ 4000 IU			p value
	Mean ± SD	Min	Max	Mean ± SD	Min	Max	Mean ± SD	Min	Max	
No of oocytes	12.4± 8.76	2	43	12.1±5.61	1	24	11.1±5.04	2	25	0.611
No of mature oocytes	9.9± 6.14	1	29	10.2±4.99	1	22	9.6± 5.56	2	25	0.732
Total oocyte score	2.2±1.66	- 2	5	2.7±1.14	0	5	1.8± 1.48	- 2	5	**0.002**

Duration of stimulation:
The duration of stimulation ranged between 8 - 13 days with an average of 10 days. Independent t test showed statistically significant association between the duration of stimulation and the oocyte score (Table 3). As the duration of stimulation increased, the dosage of gonadotropins required also increased, which yielded lower oocyte scores. (Chart 11, table 5)

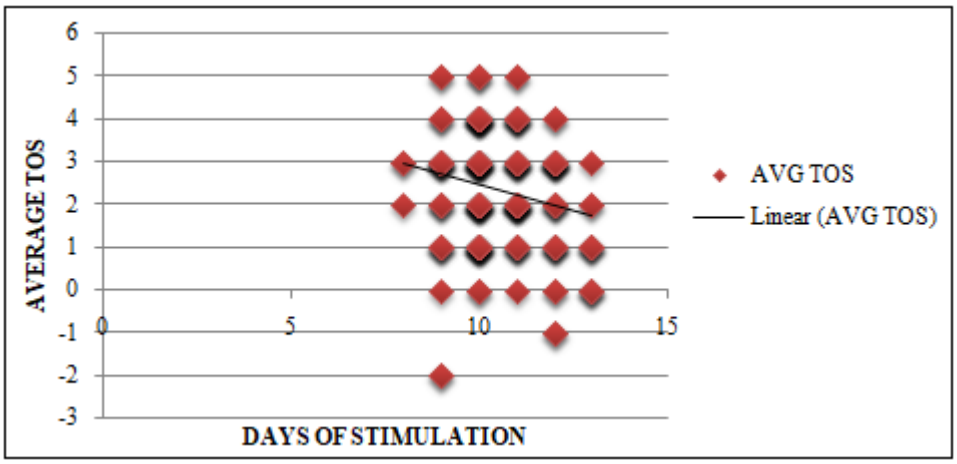


Chart 11: Scatter plot showing negative correlation between the duration of stimulation and the average TOS

4. Discussion

The effect of gonadotropins on a given oocyte is difficult to evaluate, because the outcome used in most studies is the pregnancy rate, which is influenced by many other factors like male, uterine factors. Other studies have evaluated the effect of dosing on oocyte retieval or embryo quality as a marker for oocyte quality. Whereas in our study the effect of gonadotropin dose purely on the quality of oocytes have been studied.

In our study we found a significant inverse relationship between total gonadotropin dosage and oocyte quality. Doses between 3001– 3999 IU yielded the best quality oocytes. It has been stated that high FSH doses may perturb bidirectional communication between the oocyte and the surrounding granulosa and cumulus cells and alter the kinetics of nuclear maturation, both of which are detrimental to oocyte quality [1, 2, 3]. The exact molecular mechanisms by which high FSH doses alter follicular development and endometrial receptivity are yet to be revealed with certainity and warrant further investigation.

Studies by Barash *et al.*, and Wu *et al.*, suggest that surplus of oocytes, obtained by using an increased gonadotropin dose, is invariably of lower quality with nuclear immaturity and compromised fertilization and implantation potential [4, 5]. This reinforces the concept that a certain hierarchy exists among antral follicles in their capability to respond to exogenous FSH, in which the most sensitive follicles at that time will develop with standard FSH exposure and provide the best quality oocytes [6, 7].

Gerber et al. [8] aimed to assess which factor had a greater influence on cycle outcomes - cycle length or gonadotropin dose. The author concluded that total gonadotropin dosing of >5, 000 IU was associated with lower live birth rates. The mean length of stimulation was 9.74 days, indicating that many patients received >450–500 IU gonadotropin per day. After multivariate analysis, however, the greatest contributing factor in fresh embryo transfer cycles was total gonadotropin dose. Interestingly, cycle length and total gonadotropin dose did not influence live birth rates in FET cycles.

Clark et al. [9] in his study on relationship between FSH dosing and live birth rates in ART cycles concluded that increasing FSH doses, even in low or poor responders, are detrimental to oocyte retrieval and ART outcomes. The highest number of oocytes retrieved was observed in the 1001–2000 IU FSH dose category. Secondly, ~80% of patients received a non - optimal dose, that is, a dose outside of the 1001–2000 IU FSH dose category.

The FSH dose effect was evaluated though linear regression models in different age groups by Edson Borges et al. The FSH dose didn't affect the oocyte yield in the patients ≤35 years old. A negative effect of FSH on cleavage stage embryo quality and blastocyst formation rate, for patients ≤38 years old. In patients ≥39 years old, there were no effects of the FSH doses on the outcomes, which means that a higher FSH dose is need for oocyte retrieval in older women [10]

In all the above mentioned studies live birth rates, oocyte retrieval, blastocyst conversion, embryo quality were the outcomes analysed, whereas in this study our main outcome was the quality of oocyte, not pregnancy rates, thereby eliminating the bias due to male factor and endometrial factors. Secondly we have assesed the quality of oocyte using a numerical scoring system. However the limitations of this study is that the effect of these doses on the oocyte quality in individual groups like poor responders and normal responders, <35 years and > 35 years were not analysed in the present study. We need larger prospective trials which analyse the dose effect relationship on purely the oocyte quality

5. Conclusion

Considering the current trends in ART where single embryo or blast transfer is preffered, the belief that 'the more the oocytes, the better the pregnancy outcomes' is outdated. Recruiting few oocytes that have the potential to fertilize and develop into a competent embryo with a high implantation capacity is the primary requirement, rather than striving for a more number of oocytes that do not fertilize or develop into good - quality embryos. Even in predicted poor responders – starting with standard high dose of gonadotropins due to fear of cycle cancellation should be reconsidered. ***It is the quality which matters not the quantity***

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