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Original Article

Potential impact of follitropin Alpha (Gonal_F) and maternal age on opportunity to get a male newborn

Jubran Khaleel Hassan[1*], Maysara Mohammed AbdulRidha AlBadran[2], Manal Khalid Nohi[3]

[1]Department of Clinical Pharmacy, College of Pharmacy, University of Basrah, Basrah, Iraq. [2]Department of Obstetrics & Gynecology, College of Medicine, University of Basrah, Basrah, Iraq. [3]Obstetrics Division, Al-Qurna General Hospital, Basrah, Iraq.

Correspondence: Jubran Khaleel Hassan, Department of Clinical Pharmacy, College of Pharmacy, University of Basrah, Basrah, Iraq. Jubran.Hassan@uobasrah.edu.iq

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ABSTRACT

The sex ratio, the proportion of males to females, impacts community dynamics. The reported sex ratio at birth is approximately 105/100 males to females. Ovulation-inducing agents, commonly used to treat infertility, may alter this ratio, favoring more female births. This study examines the potential influence of these medications, particularly Follitropin Alpha, and Maternal age on neonatal gender distribution. The study involved 227 pregnant women in active labor, divided into two groups: spontaneous pregnancies (N=102) and induced pregnancies (N=125). The induced group included those treated with ovulation-inducing drugs such as letrozole, clomiphene citrate, or Gonal-F. Male newborns were significantly fewer in the ovulation-induced group compared to the spontaneous pregnancy group (37.6% vs. 52%, $p<0.05$). Maternal age did not influence the sex ratio, though the impact of induction drugs declined with age. Gonal-F alone yielded a comparable male-to-female ratio to spontaneous pregnancies (55.6% vs. 52%). However, combining Gonal-F with clomiphene or letrozole significantly reduced male births to 32% compared to 52% in spontaneous pregnancies. Ovulation induction drugs (OIDs) may skew the sex ratio toward female births, independent of maternal age. While Gonal-F alone does not affect male birth likelihood, in reverse to combining it with clomiphene or letrozole significantly reduces the probability of male neonates. The effect of OIDs on the male-to-female ratio tends to diminish as maternal age increases.

Keywords: Gonadotropin, Gonal F, Maternal age, Male%

Introduction

Infertility significantly affects women's overall well-being, impacting their mental, emotional, sexual, and spiritual health. Anxiety and depression are common among women facing infertility [1, 2]. Involuntary childlessness is a public health concern that profoundly alters lives, making access to reproductive services and support essential [3]. Many individuals experiencing infertility receive assistance through medical evaluations, hormonal treatments, drugs like letrozole, clomiphene citrate, and advanced options like in vitro fertilization (IVF) [4, 5]. Follitropin alpha, marketed as Gonal-F, is a widely utilized fertility medication that has garnered extensive research attention, particularly regarding its potential influence on the male-to-female ratio in offspring [6, 7]. The sex ratio, defined as the number of males versus females in a population, is not static; it is influenced by various factors such as family size, birth order, parental age, exposure to environmental toxins, parental occupation, race, coital rate, hormonal therapies, stress, certain illnesses, and even wartime conditions [8, 9]. The World Health Organization (WHO) indicates a slight bias toward male births, with a typical ratio of approximately 105 males for every 100 females, meaning that around 51% of births result in baby boys [10]. Emerging research suggests that administering follitropin alpha may affect the male-

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to-female birth ratio [11]. While the exact mechanisms behind this effect are still being explored, some studies propose that the drug's influence on hormonal pathways and follicular development could skew the sex ratio [12, 13]. Follitropin alpha is known to regulate antral follicular growth and steroidogenesis, processes that may play a role in sex determination [14, 15]. However, the evidence on this issue remains mixed and inconclusive [16]. Some studies report a significant increase in male births following treatment with follitropin alpha, while others find no significant impact on the sex ratio [17-20]. Many studies investigated the effect of letrozole, clomiphene, and gonadotropins (Gonal- F) individually, and there is no study has reported the effect of the combination of these drugs on the gender ratio.

Aims

To determine if ovulation induction by follitropin Alpha (Gonal_F) with or without other ovulation induction drugs like clomiphene and letrozole affects neonatal gender ratio, also determine whether maternal age has an effect on % of neonatal gender

Materials and Methods

This study is a retrospective case-control study that was conducted in Al-Mawany and Al-Qurna hospitals in Basra-Iraq through a period extending from April 2022 to May 2023.

Data were collected from pregnant women attending these hospitals in active labor, and verbal, in addition to a written informed consent, was obtained from all participants. A total of 227 women (age range: 18 -48 years) have been enrolled in the study, and they have been divided into two groups according to whether they conceived spontaneously or received ovulation induction drugs. Group I (125) comprised women who conceived after receiving ovulation induction medication within the two menstrual cycles preceding conception (case group), while group II (102) comprised women who conceived spontaneously (control group).

Detailed information was elucidated from all participants, including age, gravidity, parity, and husband age. In a group I more information was obtained about the indication for ovulation induction (only in 28 women the cause was unexplained infertility, in the rest, it was anovulation or oligo-ovulation), drugs and regimens used for ovulation induction and the duration of treatment so women in a group 1 where subdivided into further subgroups according to agent they received.

Induction pills subgroup (N=32)

These women received 100 mg of clomiphene citrate or letrozole 5mg for five days, from day 2 till day 7 of spontaneous or induced menstruation.

Gonadotropin subgroup (N=18)

Women in this subgroup got pregnant after receiving recombinant FSH follitropin Alpha (Gonal-F)®

The regimen used for most is the modified step-up protocol: 75 IU of gonadotropin is administered daily over 7–14 days. If no follicle is > 10 mm from the initial dose, it is increased by 37.5 IU. If estradiol levels are > 200 pg/ml or follicles are > 10 mm, then the same dose is continued until ovulation is triggered by 5000 IU OF Human Chorionic Gonadotropin(HCG)

Combination of gonadotropin and induction pills group (N=75)

Received the usual regimen of clomiphene or letrozole with added 75 IU of Gonal-F®, 3 doses starting from day 7 of the menstrual cycle and on alternate days.

Exclusion criteria

Neither pregnant nor conceived by intrauterine insemination (IUI), nor in vitro fertilization (IVF) were included in the study. Similarly, multiple pregnancies were excluded from the study. Involved women were followed till delivery to identify the gender of the neonates.

Statistical analysis

Data obtained from the present study were analyzed statistically using Medcalc® V.12 . Continuous data were expressed as mean ± SD, whereas categorical data were expressed as count and percent. Student *t*-test was used to compare continuous data, whereas Chi- and Fisher exact tests were used to compare categorical data . P-value less than 0.05 was considered statistically significant. The Total Sample size was evaluated by G. power V3.1® to be N=220, and the sample was collected according to the time designed for this study, as one year, and the Total sample N=227

Post hoc Analysis by Cochran-Armitage Trend Test was performed to determine if induction drugs (induction subgroups) have a significant effect when compared with the control group.

Results and Discussion

Table 1 shows the data for the studied women. No significant difference was found among the ages of the parents in both groups, which is important to eliminate the possible effect of parental age on neonatal sex. Women who conceived after receiving ovulation induction were of lower parity and gravidity than those who conceived spontaneously (1.8 ± 1.8 vs. 3.2 ± 2.2 for parity and 3.1 ± 2.1 vs. 4.7 ± 2.6 for gravidity).

Table 1. Data of patients who participated in the study

Parameters	Spontaneous pregnancy N= 102	Pregnancy by induction N=125	P-value
Husband Age (years)	35 ± 7	33.3 ± 8.1	0.094
≤25	12 (11.8%)	29(23.2%)	
26-35	39 (38.2%)	38(30.4%)	0.0670
>35	51(50.0%)	58(46.4%)	
Wife age	31.2 ± 6.9	29.7 ± 7.5	0.123
≤25	26(25.5%)	44(35.2%)	
26-35	47(46.1%)	55(44.0%)	0.2100
>35	29(28.4%)	26(20.8%)	
Average parity	3.2 ± 2.2	1.8 ± 1.8	**<0.0001**
Average gravidity	4.7 ± 2.6	3.1 ± 2.1	**<0.0001**
Age difference between husband and wife	3.8 ± 2.8	3.8 ± 3.8	0.896
male %	53(52.0%)	47(37.6%)	**0.0305**

P value <0.05 is considered significant.

Table 2 There was no significant effect of the age of the mother on gender ratios in spontaneous and induced pregnancy groups when comparing the spontaneous to the induced pregnancy group. But noticeably, in the induced pregnancy group, the male /female ratio is steadily increasing as the advancing age of the mother (0.42 for mothers with age <25 to 0.86 for mothers with age >35years, which is clear in the **Figure 1** and may reflect ameliorating effect of age of mother to the effect of induction drugs on male / female ratio.

Table 2. Shows the association between the age of the mother, % of males, and the male-to-female ratio in both spontaneous and induced pregnancy groups.

Parameters	Male	Female	Male /female ratio	Male %	P-value
		Spontaneous pregnancy N= 102			
≤25	11	15	0.73	42.3%	
26-35	27	20	1.35	57.4%	0.4635
>35	15	14	1.07	51.7%	
		Induced pregnancy N=125			
≤25	13	31	0.42	29.5%	
26-35	22	33	0.67	40.0%	0.3392
>35	12	14	0.86	46.2%	

P-value <0.05 is considered significant

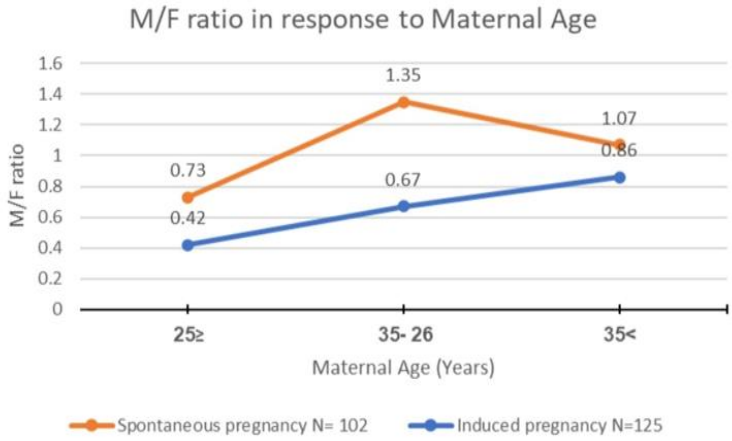

Figure 1. Show changes oof male to female ratio for new born in both spontaneous induction groups. In induction group the the effect of induction drugs on male / female ratio is reduced maternal get older

Table 3 indicates gender percentages of neonates delivered after pregnancy induced by using different drugs were compared with those of spontaneous pregnancy. There was a significant difference in the Male % and male-to-female ratio in the

subgroups of induced pregnancy as compared to the group of spontaneous pregnancy.

Table 3. shows a comparison of Neonatal gender distribution among the control group (spontaneous pregnancy) and the ovulation induction subgroups (induction pills, follitropin alpha, and combination of (pills & follitropin alpha)

Parameters		N	Female	Male	Male%	Male/female ratio	P-value
Spontaneous pregnancy		**102**	49	53	52%	1.08	
Induced pregnancy subgroups	Induction pills (Clomiphene or letrozole)	**32**	19	13	40.6%	0.68	**0.043**
	Gonal-F (follitropin alpha)	**18**	8	10	55.6%	1.25	
	Gonal-F (follitropin alpha) & induction Pills	**75**	51	24	**32%***	0.47	
Induced Pregnancy Group **(total)**		**125**	78	47	**37.6%[a]**	0.6	

P-value <0.05 is considered significant.
* Significant as compared with the Spontaneous pregnancy group, the Comparison was evaluated by the Cochran-Armitage Trend Test
[a] Significant difference with the Spontaneous pregnancy group using chi-square analysis

Using induction pills (clomiphene or letrozole) significantly lowers the opportunity to get a male neonate to 40.6% as compared to 52 % for spontaneous pregnancy.

When Gonal F (Follitropin Alpha) is used alone, the male/Female ratio and Male % may reach to level of spontaneous pregnancy or slightly higher (55.6% vs 52% for spontaneous pregnancy), as in **Table 3**.

In reverse, when a combination of pills with gonal F is used, the opportunity to get a male neonate is sharply and significantly declined to reach 32% vs 52% for spontaneous pregnancy.

The ratio between the number of males to females in a society is known as the sex ratio; this ratio has an impact on the community. At birth, the 'natural sex ratio' is about 105 (ranging from 103 to 107) males per 100 females [21-23]. In some societies, including ours, there is some parental preference for one neonatal sex over the other (mainly boys), so factors that may affect neonatal sex are of interest. Nowadays, ovulation-triggering agents are widely used for anovulatory infertility or unexplained infertility, with a probable impact on neonatal gender. We carried out this study to determine whether this effect is possible and which drugs used (as individual drugs or as a combination) may have a greater effect, and whether maternal age may also be involved in this effect.

According to our study, there is no association between maternal age and neonatal sex; this result is consonant with that of Eliana Ein-Mor *et al*. [24, 25]. However, it disagrees with other studies that suggest increasing parental ages decrease the sex ratio, explaining for that by an increase gonadotrophin concentration with age in female and dropping in androgen in male, which affect the ratio of Y- and X-bearing spermatozoa [26-28]. However, analysis of the fractions of X- and Y-bearing spermatozoa in samples of semen was not significantly affected

In our study, the overall M/F sex ratio among women who conceived following ovulation induction is lower than that of women who conceived spontaneously (37.6% vs. 52% for spontaneous pregnancy), mainly after the consumption of a combination of pills with gonadotropin (32% vs. 52% for spontaneous pregnancy). Our result is in agreement with that of J.H. Sampson [31, 32]. James proposed that the percentage of female births was significantly higher when natural insemination occurred on the day of presumed ovulation, and more male births when insemination occurred 6 days before ovulation because of high maternal gonadotropin concentration around the time of conception [33, 34]. However, the trend was the opposite when artificial insemination was practiced [35]. However, these theories had been refuted by other studies that found no correlation between the timing of intercourse or the use of ovulation induction and sex ratio [36, 37]. Other explanations are that variations in vaginal and intracervical pH and cervical mucus consistency in women receiving ovulation-triggering agents, mainly clomiphene citrate, may result in selective transport of spermatozoa [31]. Adequate cervical mucus is crucial for sperm motility and transport to the uterine cavity. Clomiphene citrate's antiestrogenic action can compromise cervical mucus receptivity to sperm, potentially affecting fertility [38]. Gonal-F alone may increase the male to female ratio to the level of spontaneous pregnancy or slightly higher (55% vs. 52% for spontaneous pregnancy); this is in agreement with the findings of Ben *et al*. [39].

Probably, there is a strong association between follitropin alpha administration and increased serum levels of estradiol, inhibin A, and inhibin B. [40, 41]. Follicle-stimulating hormone (FSH) tends to rapidly elevate inhibin B levels in females, without exerting an effect on activin [42]. While some studies suggest a possible link between inhibin B levels and male fetal development, the exact nature of this relationship is still under investigation. Inhibin B levels follow a distinct pattern throughout the menstrual cycle. They peak during the early to mid-follicular phase, decrease in the late follicular phase, and experience a brief rise alongside the luteinizing hormone (LH) surge. However, inhibin B levels then drop significantly about seven days after ovulation. This pattern suggests that the corpus luteum, which forms after ovulation, does not produce inhibin B. [43].

We hypothesize that the administration of external FSH (Gonal-F), which has a prolonged terminal half-life (up to 59 hours) [44, 45], may lead to a persistent elevation in serum FSH levels. This elevation could disrupt the normal rhythm of inhibin B secretion, resulting in prolonged elevated levels of inhibin B, followed by increased estrogen levels [46] throughout the menstrual cycle, thereby creating conditions favorable for male fetus conception. Higher inhibin B levels may enhance the environment for Y-

bearing sperm, thereby increasing the likelihood of male embryo development [47]. Conversely, the addition of letrozole or clomiphene citrate may affect circulating estrogen levels [48], potentially disrupting this favorable environment and reducing the chances of male embryo development. However, this hypothesis requires further validation.

Conclusion

- Ovulation induction medications (OIDs) can potentially influence the sex ratio of offspring, favoring female births.
- Clomiphene citrate and letrozole are OIDs that have been specifically associated with increased female births.
- Follitropin Alpha, like Gonal-F, when used alone, does not seem to reduce the likelihood of male offspring.
- The use of follitropin alpha in conjunction with clomiphene or letrozole could potentially decrease the probability of a male offspring.
- OIDs' effects on the male-to-female ratio tend to diminish as maternal age increases.
- Maternal age, without (OIDs), however, appears to have no significant impact on this outcome.

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