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A COMPARATIVE CLINICAL EVALUATION IN NATURAL VERSUS STIMULATED IVF CYCLES EMBRYO

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INTRODUCTION: The impact of controlled ovarian stimulation (COS) on oocyte and subsequent embryo quality remains controversial. In the present study we have compared embryo quality in natural and stimulated cycles in the same group of patients.

METHODS: This retrospective study was comprised of patients with a regular menstrual cycle who had IVF after COS using rFSH in a long GnRH agonist protocol. In all stimulated cycles the patients had fresh embryos transferred and surplus good quality embryos cryopreserved. Subsequently the same patients were treated with a modified FER cycle (mFER) where thawing of the frozen embryos was combined with aspiration of the dominant follicle in the natural cycle. The embryo cleavage stage and quality score were compared between the stimulated and the natural cycle for the patients having an embryo in the natural cycle**.**

RESULTS: In 177 cases patients returned for mFER in a natural cycle. Spontaneous ovulation had occurred in 35 cycles. In 17 cycles no oocyte was retrieved at aspiration and in 125 cycles 128 oocytes were aspirated. In the stimulated cycles from these patients we had obtained 950 embryos (cleavage rate 70.4%) versus 85 embryos (cleavage rate 66.4%) (P = 0.34) in the natural cycles. Comparing the embryos in the natural and stimulated cycles in all patients having an embryo in the natural cycle, we found no difference in the distribution between the different cleavage stages. Of the cleaved embryos, 53% in the stimulated cycles had >4 cells versus 59% in the natural cycles after 2 days culture (P = 0.31). In the stimulated cycles 61% of the embryos had <10% fragmentation at the time of transfer on day 2, compared to 69% in the natural cycles (P = 0.15).

CONCLUSION: The administration of exogenous gonadotrophins was not reflected in cleavage capacity or quality assessment of the resulting embryos.

Key words: *Developmental potential/embryo quality/impact of gonadotrophins/in vitro/in vivo*

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INTRODUCTION AND LITERATURE REVIEW

Limited information is available on the impact of gonadotrophin stimulation on the quality of oocytes and embryos. In mice, increased pre- and post-implantation mortality and fetal growth retardation have been observed in superstimulated animals (Ertzeid and Storeng, 1992). A case report by Akagbosu et al. (1998) and a paper by Aboulghar et al. (1997) suggest a negative effect of ovarian hyperstimulation syndrome on the quality of the oocytes. In a recent study Ng et al. (2003) found no negative impact on the quality of the oocytes and embryos in patients with excessive ovarian response during controlled ovarian stimulation (COS) compared to patients with a more moderate response. However, no comparison was made to embryos from natural cycles.

A number of studies have previously compared stimulated and unstimulated IVF cycles (Levy et al., 1991; Svalander et al., 1991; Paulson et al., 1992; Claman et al., 1993; MacDougall et al., 1994; Lindheim et al., 1997; Ingerslev et al., 2001; Ng et al., 2001). The studies report very different results with implantation rates varying from 0 to 33% in the natural cycles and from 7 to 24% in the stimulated cycles. For review see Pelinck et al. (2002).

In this retrospective study we have utilized a previously described (Kim et al., 1996) treatment modality, modified frozen embryo replacement (m-FER), in which we aspirate and fertilize the oocyte from the leading follicle when monitoring the woman's natural cycle for correct timing of the FER treatment.

This means that we can compare the developmental capacity and embryo quality in a pool of embryos originating from oocytes aspirated in the same cohort of women, once at the occasion of an ovarian stimulation and once at the time of spontaneous ovulation in the same woman.

The aim of this study was to compare the morphology and early cleavage stages of the embryos from stimulated and natural cycles in the same woman in order to assess any effect of COS on early embryo development. Thus in backdrop the aim of present research paper is "***A COMPARATIVE CLINICAL EVALUATION IN NATURAL VERSUS STIMULATED IVF CYCLES EMBRYO"***

MATERIALS AND METHODS

PATIENTS

This retrospective analysis includes 177 couples undergoing IVF at authors word desitination Zulekha Hospital, Sharjah, UAE. The patients were referred to IVF due to tubal infertility or unexplained infertility. The study represents a consecutive series of patients returning for a modified FER cycle after a stimulated treatment cycle. The study was approved by all legal formalities.

Stimulated cycles

All patients were treated with a long protocol using GnRH agonist (Suprefact®, Hoechst Marion Roussel; or Synarela, Phamacia) as down-regulation for desensitization from day 21 of the cycle onwards and recombinant (r)FSH (Gonal-F®, Serono; or Puregon®, Organon) for COS. hCG (Profasi®, Serono) was given 36 h before oocyte retrieval. An average of 12.3 6 4.9 follicles were aspirated. While only clearly fertilized embryos (two pronuclei) were transferred, the data in this study may include a few cases with inconclusive assessment of fertilization. Embryo replacement was performed after 48 h.

Modified FER cycles

In the natural cycles we aspirated and fertilized the oocyte from the leading follicle when monitoring the woman's cycle for correct timing of the FER cycle. The aspiration was performed when the dominant follicle was >17 mm in diameter. hCG (Profasi®; Serono) was given 36 h before oocyte retrieval.

Embryo culture

The oocytes were cultured in 4-well dishes (Nunc) in 0.5 ml standard IVF medium (Universal IVF media; Medicult). After 4 h of culture, insemination was performed by addition of $0.15X10^6$ sperm cells. Embryo evaluation was performed on the morning of day 2 after insemination.

Embryo evaluation

Both in the stimulated and in the natural cycles the embryos were evaluated for cleavage stage and scored for morphology prior to transfer, in accordance with previously described criteria (Staessen et al., 1990; Ziebe *et al*., 1997). Briefly, the scoring system was: 1.0: equally sized symmetrical blastomeres without fragmentation; 2.0: unevenly sized blastomeres without fragmentation; 2.1: embryos with <10% fragmentation; 2.2: embryos with 10-20% fragmentation; 3.0:

embryos with 20-50% fragmentation; 4.0: embryos with >50% fragmentation.

STATISTICAL ANALYSIS

Statistical analysis was done by the χ^2-test. Values was considered significant if $P < 0.05$.

RESULTS

The study included a total of 177 stimulated cycles and 177 subsequent natural cycles (Table IA). In 35 natural cycles ovulation had taken place prior to aspiration. In another 17 natural cycles no oocytes were aspirated. In 125 natural cycles oocytes were aspirated (Table IA).

In the stimulated cycles from women with oocytes in the natural cycle, a total of 1349 oocytes were aspirated (10.8 ± 4.7 oocyte/cycle). In the natural cycles 128 oocytes were aspirated in 125 cycles (Table IB). The average age of these women was 32.8 ± 3.2 at the time of the stimulated cycle and 33.6 ± 3.2 when they returned for the m-FER cycle.

No significant difference was found in cleavage rate between oocytes from stimulated and natural cycles. In the stimulated cycles we obtained 950 embryos (cleavage rate 70.4%), versus 85 embryos (cleavage rate 66.4%) in the natural cycles (P = 0.34) (Table IB).

Table I.

	Stimulated cycles	Natural cycles
Table I A. All patients		
No. of included patients	177	177
Mean age of the women (years)	32.8 ± 3.3	33.6 ± 3.3
Average total FSH dose in the stimulated cycle (IU)	1.881 ± 807	-
Average days of stimulation	10.4 ± 2.6	-
Average endometrial thickness (mm)	11.0 ± 7.8	8.2 ± 1.9
Day of hCG	11.4 ± 1.9	13.2 ± 2.2
No. of cycles	177	177
Ovulation prior to aspiration	0	35
No. of cycles with no oocyte retrieved	0	17
No. of cycles with oocytes	177 (100)	125 (71)
Table I B. Patients with oocytes in the natural cycle		

No. of cycles with oocytes	125	125
Mean age of woman at treatment (years)	32.8 ± 3.2	33. ± 6 3.2
No. of oocytes retrieved	1349	128
No. of embryos	950	85
Cleavage rate (%)	70.4	66.4, *P* = 0.34
Table I C. Patients with embryos in natural cycle		
No. of cycles with embryos	85	85
Mean age of woman at treatment (years)	32.8 ± 3.4	33.7 ± 3.3
No. of oocytes	920	85
No. of embryos	670	85
No. of embryos with >4 cells	355 (53)	50 (59), *P* = 0.31
No. of embryos with <10% fragmentation	411 (61)	59 (69), *P* = 0.15

Values in parentheses are percentages.

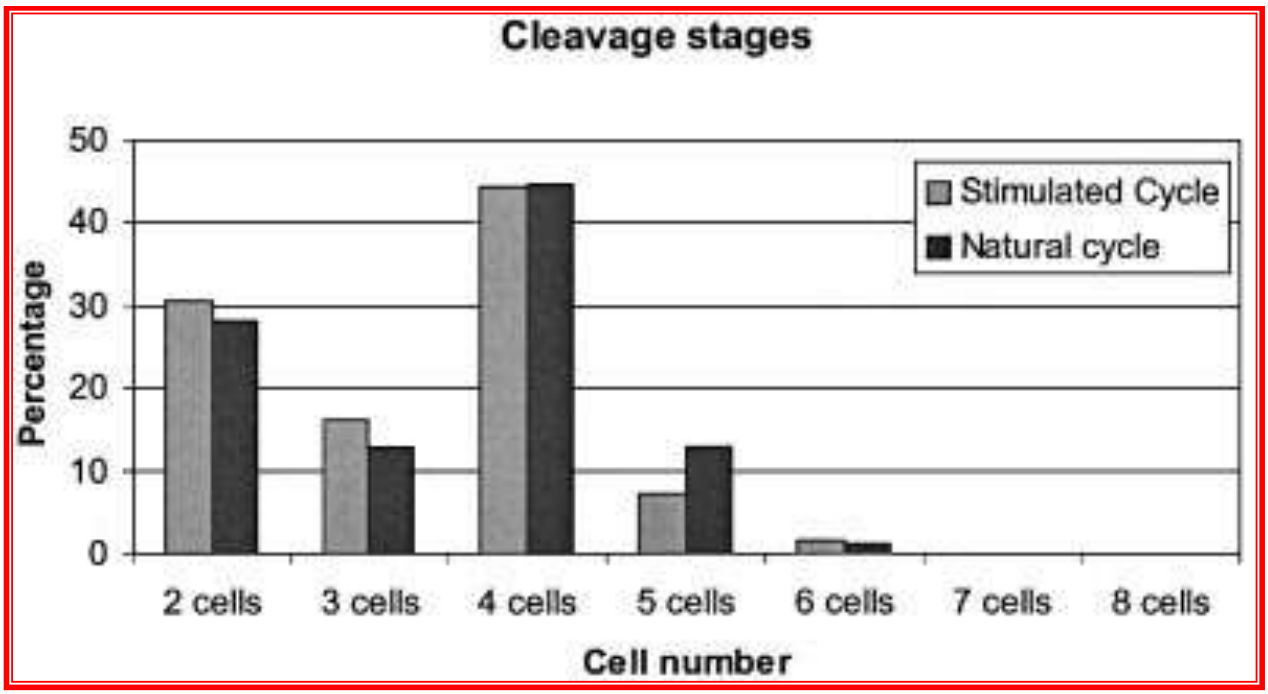

Figure 1. Cleavage stage of all embryos after 2 day culture of oocytes from stimulated and natural cycles in patients having an embryo in the natural cycle.

In order to perform a relevant comparison of embryo cleavage stage and quality score between the stimulated and natural cycles, we have compared all patients having an

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embryo in the natural cycle. The mean age of the women in this subgroup was 32.8 ± 3.4 years in the stimulated cycle and 33.7 ± 3.3 years in the natural cycle (Table IC). The distribution between the different cleavage stages was the same in both groups (Figure 1). After 2 days of culture 44.3% of the embryos had cleaved to the 4-cell stage in the stimulated cycles versus 44.7% in the natural cycles. Furthermore, 53% of the embryos in the stimulated cycles had >4 cells at day 2 compared to 59% in the natural cycles (*P* = 0.31) (Table IC).

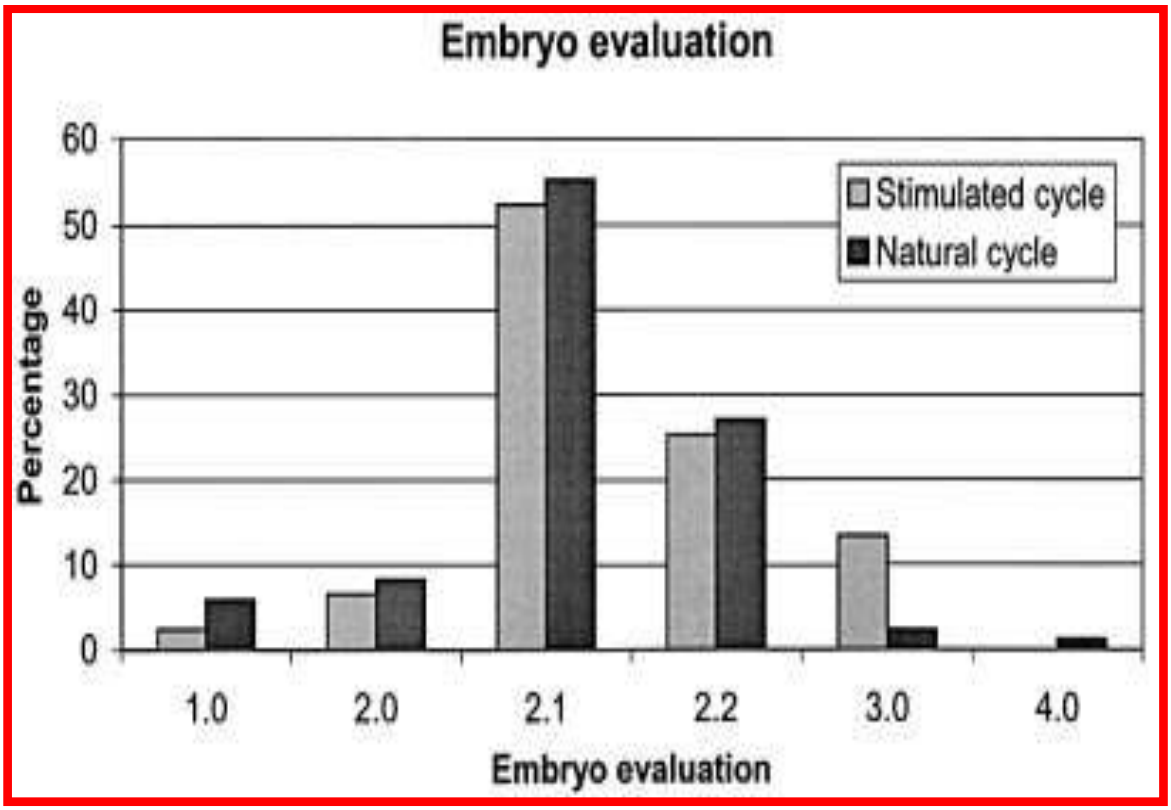

Figure 2. Evaluation of all embryos after 2 day culture of oocytes from stimulated and natural cycles in patients having an embryo in the natural cycle.

We found no difference in the fragmentation pattern between the stimulated and natural cycles (Figure 2). In the stimulated cycles, a total of 61% of the embryos had <10% fragmentation at the time of transfer, compared to 69% in the natural cycles (P = 0.15) (Table IC).

DISCUSSION

The data from the present retrospective analysis suggest that the

administration of recombinant FSH after pituitary desensitization is not reflected in the morphology of the early stages of embryonic development. Neither the cleavage rate of the oocytes nor the early cleavage stage morphology assessment of the embryos differed between the stimulated cycles and the natural cycles.

These findings suggest that the only reason why hormonal stimulation increases the likelihood of achieving a pregnancy is by increasing the number of oocytes retrieved which again might provide us with more embryos to select from at the time of transfer.

In theory, the hormonal environment in the natural cycle provides optimal conditions for the maturing follicle and oocyte. Indeed cycles with desensitization followed by stimulation with gonadotrophins differs markedly from the natural cycle. In a study by Kaneko et al. (2000), COS was shown to have an impact on apoptosis in the granulosa cells.

During IVF we are often faced with pronounced embryo heterogeneity both in terms of morphology (Cummins *et al.,* 1986; Puissant *et al.,* 1987; Claman *et al.,* 1987; Staessen *et al.,* 1992; Steer *et al.,* 1992; Shulman *et al.,* 1993; Giorgetti *et al.,* 1995; Ziebe *et al.,* 1997; Hardarson *et al.,* 2001; Van Royen *et al.,* 2001) and chromosomal constitution (Ziebe *et al.,* 2003; Johansson *et al.,* 2003). The reason for this diversity is largely unknown but it reflects to some degree the developmental capacity of the embryo. Several underlying factors are probably contributing to the diversity including the in vitro environment and culture conditions. In a previous study Van Blerkom and Davis (2001) demonstrated the effect of repeated ovarian stimulation in mice, resulting in a significant increase in the frequency of spindle defects resulting in chromosomal errors with each series of ovarian stimulation.

We have no directly measurable parameters of oocyte quality except for the nuclear maturity. However, an indirect indication of the oocyte quality could be the developmental capacity.

Despite the fact that no difference was found in this study concerning the light microscopic morphological appearance of the embryos, there may still be an effect as a result of administration of gonadotrophins on embryo quality not reflected until

implantation or even later. The advantage of our study design was that the stimulated and the natural cycles were in the same patients. However, the study design with stimulated versus natural cycles resulting in many versus one embryo in each woman this retrospective study may result in insufficient power to detect a minor impact on embryo development.

A disadvantage of the study design is that we were unable to assess the implantation rate of the natural cycle embryos separately, since in the majority of cases they were transferred in combination with the thawed embryos.

In conclusion, we find that the use of hormonal stimulation in assisted reproductive treatment does not alter the ability of the oocyte to cleave after fertilization. Furthermore, the developmental capacity, defined as the number of blastomeres after 2 days of culture, is unaffected of the use of exogenous gonadotrophins. Finally, embryo quality in terms of degree of fragmentation is similar between embryos resulting from stimulated cycles and natural cycles.

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