SHORT COMMUNICATIONS

COPENHAGEN, DENMARK

Progesterone-Activated Spermatozoa Used for In Vitro Fertilization

Submitted: September 29, 1997
Accepted: October 31, 1997

INTRODUCTION

Recent studies have shown that progesterone from follicular fluid or progesterone produced locally by the cumulus cells stimulates the spermatozoa in the female reproductive tract and enhances the fertilizing potential (reviewed in Refs. 1–3). Through specific membrane receptors progesterone induces intracellular calcium release, which results in hyperactivation and primes the spermatozoa rapidly to undergo the acrosome reaction on making contact with the zona pellucida (4–6). In most assisted reproduction programs enhancement of the fertilizing capacity of spermatozoa by natural inducers such as progesterone is usually not undertaken. The aim of the present study was to evaluate whether progesterone-activated spermatozoa from men with normal semen resulted in an increased cleavage rate and/or preembryo quality in connection with IVF treatment.

MATERIALS AND METHODS

Fifteen women (34.5 ± 1.1 years, mean $\pm$ SE; range, 28–43 years) undergoing IVF treatment were included in this study (tubal infertility, 9; endocrine abnormality, 5; idiopathic infertility, 1). The hormonal treatment consisted of downregulation with gonadotropin-releasing hormone agonist (GnRHa; Suprefact, Hoechst, Copenhagen Denmark), 0.5 mg s.c. daily for a minimum of 14 days, starting in the midluteal phase of the cycle. When downregulation was confirmed by a quiescent ovary as judged by ultrasound, the dose of

GnRHa was reduced to 0.2 mg s.c. daily until the day of human chorionic gonadotropin (hCG) injection. Ovarian stimulation consisted of 150–450 IU follicle-stimulating hormone (FSH; Gonal F; Serono, Copenhagen, Denmark) per day until at least two follicles with a diameter (i.e., mean of two measurements) exceeding 17 mm were observed, when an ovulatory dose of 10,000 IU hCG (Profasi, Serono) was given by i.m. injection. Oocyte pickup was performed 36 hr later, on cycle day 14.4 ± 0.4 (range, 13–17). The mean number of ampoules (i.e., 75 IU per ampoule) used for ovarian stimulation was 33 ± 4 (range, 27–54).

Oocytes were prospectively and randomized allocated to receive either progesterone-activated or normal purified spermatozoa. For each woman half of the oocytes received progesterone-activated spermatozoa and the other half normal purified spermatozoa; each woman thereby serves as her own control. A mean of 14 ± 3 (range, 3–25) oocytes was retrieved from each woman. Each oocyte was inseminated with 100,000 motile spermatozoa regardless of the purification method. After 2 days in culture, cleavage and preembryo quality were evaluated. Preembryo development was defined as oocytes that fertilized and cleaved into at least two-cell preembryos.

Only men with normal semen parameters according to WHO criteria were included in this study. The semen samples contained a total of $151 \pm 43 \times 10^6$ (range, $40\text{--}450 \times 10^6$) spermatozoa, the percentage motile was $65 \pm 4\%$, and the motility score was 3.5 (scale, 0–4). The mean age of the men was 39.2 ± 3.0 years (range, 30–49 years). All semen samples were purified using routine Percoll gradient centrifugation (55 and 80% Percoll) (7), which did or did not contain progesterone (2 $\mu\text{g}/\text{ml}$). Thus, progesterone activation of the spermatozoa was performed simultaneously with Percoll purification. The efficacy of this procedure has been described previously (8). Purification of spermatozoa with and without progesterone activation was performed simultaneously and during the same run of the centrifuge.

The study was approved by the Central Ethical Committee of Denmark.

Table I. Progesterone-Activated Spermatozoa Used in Connection with IVF in 15 Women^a

	No. oocytes	No. embryos	No. blastomeres				Quality score				
			2	3	4	5	1.0	2.0	2.1	2.2	≥3
+ Progesterone	104	58 (56%)	41	6	10	1	7	23	21	4	2
– Progesterone	107	60 (56%)	38	10	9	3	9	22	21	3	1

^aNo difference between pregnant ($n = 5$) and nonpregnant ($n = 10$) women.

The frequency at which the oocytes were fertilized and developed into embryos was compared using Student's *t*-test for paired observations after log transformation. Comparison of the number of blastomeres and the quality score between the two types of spermatozoa were performed using ANOVA.

RESULTS

The results are summarized in Table I. Overall a total of 211 oocytes resulted in the formation of 118 preembryos (56%). In the group of oocytes inseminated with normal purified spermatozoa, 60 preembryos (56%) developed from 107 oocytes, which was similar to the rate in the group of oocytes receiving progesterone-activated spermatozoa, in which 104 oocytes resulted in the formation of 58 preembryos (56%) ($P > 0.10$). There was no difference between the two groups with regard to the number of blastomeres of each preembryo or with regard to the quality score of the preembryos ($P > 0.10$). There was no statistical significant difference between those who became pregnant ($n = 5$) and those who did not ($n = 10$) with regard to any of the monitored parameters ($P > 0.10$).

DISCUSSION

There is now good evidence to suggest that progesterone or progesterone-related steroids in synergy with the zona pellucida are natural inducers of the acrosome reaction and play an important physiological role in the process of fertilization (reviewed in Refs. 1–3). Furthermore, progesterone has also been shown to enhance sperm motility (5,8,9), and because the spermatozoon cannot penetrate the zona pellucida unless it is hyperactivated (3), it may be assumed that fertilization is affected by a proper interaction between the spermatozoon and the progesterone-rich follicular fluid released to the oviduct at ovulation. Despite the aforementioned considerations and the fact that the present method of progesterone activation has been shown sig-

nificantly to enhance sperm motility, the present study was unable to demonstrate any beneficial effect of progesterone activation in connection with IVF treatment. One possible explanation may be that the spermatozoa were obtained from men with normal semen and that Percoll-purified normal spermatozoa naturally contain a sufficient number of hyperactivated spermatozoa to leave the rate of fertilization unaffected. Therefore, the results of the present study obviously limit the usefulness of progesterone-activated spermatozoa in routine IVF treatment but do not exclude that a beneficial effect may be seen with spermatozoa from men with decreased semen quality such as oligozoospermia. We are now planning to perform a study to elucidate whether the fertilizing potential of suboptimal sperm samples can be improved via progesterone activation.

REFERENCES

1. Aitken RJ: Molecular mechanisms regulating human sperm function. *Mol Hum Reprod* 1997;3:169–174
2. de Lamirande E, Leclerc P, Gagnon C: Capacitation as a regulatory event that primes spermatozoa for the acrosome reaction and fertilization. *Mol Hum Reprod* 1997;3:175–194
3. Breitbart H, Spungin B: The biochemistry of the acrosome reaction. *Mol Hum Reprod* 1997;3:195–202
4. Blackmore PF, Beebe SJ, Danforth DR, Alexander N: Progesterone and 17 α -hydroxyprogesterone. Novel stimulators of calcium influx in human sperm. *J Biol Chem* 1990; 265:1376–1380
5. Revelli A, Modotti M, Piffaretti-Yunez, Massobria M, Balerna M: Steroid receptors in human spermatozoa. *Hum Reprod* 1994;9:760–766
6. Sueldo CE, Oehninger S, Subias E, Mahony M, Alexander NJ, Burkman LJ, Acosta AA: Effect of progesterone on human zona pellucida sperm binding and oocyte penetrating capacity. *Fertil Steril* 1993;60:137–140
7. Ziebe S, Yding Andersen C: Isolation of motile spermatozoa: Comparison of Percoll centrifugation, sperm prep filtration, and swim-up techniques. *J Assist Reprod Genet* 1993;10:485–487
8. Yding Andersen C, Jørgensen N: Improvement of sperm motility by the addition of progesterone to the Percoll medium during sperm purification. *Hum Reprod* 1995;10:3183–3185
9. Uhler ML, Leung A, Chan SY, Wang C: Direct effects of progesterone and antiprogesterone on human sperm hyperactivated motility and acrosome reaction. *Fertil Steril* 1992;58: 1191–1198

Claus Yding Andersen¹

Laboratory of Reproductive Biology
The Juliane Marie Center for Children, Women and
Reproduction
University Hospital of Copenhagen, Section 5712
Blegdamsvej 9
DK-2100 Copenhagen, Denmark

Jørgen Grinsted

The Fertility Clinic Trianglen
Østerbrogade 62
DK-2100 Copenhagen, Denmark

¹ To whom correspondence should be addressed.