RGD-binding integrins were not involved in the gamete fusion process.

Conclusion: In humans, the surface expression of β_1 integrin varies during oocyte maturation but its level of expression at each of the germinal vesicle, MI and MII stages is not correlated with the extent of gamete fusion. Therefore we suggest that β_1 integrin participates in the human sperm–egg interaction but is not mandatory for fusion. Human gamete fusion may involve other co-factors that do not belong to the RGD-binding integrin family, but these are still unknown.

17.30–17.45

O-135. Is IUI for male subfertility based on any evidence?

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Introduction: IUI and ovarian stimulation are still extensively applied treatment modalities for male subfertility. However, the efficacy of IUI with or without ovarian stimulation remains a matter of debate. Differences found in individual trials have not always reached statistical significance. One reason might be the lack of power because of the small number of participants. Therefore, it seemed appropriate to perform a meta-analysis combining the results of available randomized controlled trials only.

Materials and methods: We used the database from the Subfertility Cochrane Centre in combination with three different computerized search programs (Medline, Embase and SCIsearch) to identify randomized controlled trials. Male subfertility had to be the only identifiable cause of subfertility and was defined as semen quality not meeting the WHO criteria for normality. Each study had to compare the efficacy of IUI with timed intercourse in natural or stimulated cycles or the efficacy of ovarian stimulation compared with no stimulation in timed intercourse or IUI cycles. The pregnancy rate per completed cycle was the main outcome of interest. Two reviewers independently selected all trials, assessed the quality of each individual trial and extracted the data. Odds ratios (OR) with 95% confidence intervals (CI) were calculated for each individual trial and, if appropriate, pooled to calculate the overall combined OR. Heterogeneity was tested using the Breslow–Day method.

Results:

Preliminary results using pooled data from randomized controlled trials

Comparisons	No. of randomized controlled trials	Peto OR (95% CI)
Natural cycle: IUI versus timed intercourse	6	3.05 (1.49–6.27)
Stimulation cycle: IUI versus timed intercourse	6	2.14 (1.30–3.51)
IUI: stimulation versus natural cycle	4	1.84 (1.01–3.34)
IUI/stimulation cycle versus timed intercourse/natural cycle	2	6.23 (2.35–16.52)

Conclusion: In male subfertility IUI significantly improves the probability of conception in both natural and stimulated cycles. Ovarian stimulation seems to improve further the efficacy of IUI, although these results should be interpreted with care because of the heterogeneity between the different trials.

17.45–18.00

O-136. Use of mini-Percoll and swim-up sperm preparation techniques in separating spermatozoa with chromatin packaging anomalies

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Introduction: The very highly packed nature of human sperm chromatin is principally due to dramatic modifications of the nucleoprotein components, the most essential consisting of the replacement of histones by protamines and further stabilization by the formation of disulphide cross-links among the cysteine residues of the protamine molecule. The aim of this study was to determine the efficiency of the mini-Percoll and swim-up sperm preparation techniques to eliminate spermatozoa with anomalies in chromatin packaging.

Materials and methods: Once collected, one portion of the semen sample was washed in PBS, fixed using 3:1 methanol/acetic acid and spread on a slide. The remainder was prepared using the swim-up or mini-Percoll technique. When prepared by the swim-up technique, the sediment and upper layer were fixed; only the final sperm wash was fixed when using the mini-Percoll technique. The spermatozoa were then stained using two fluorochromes: monobromobimane (MMBr), which indicates the presence of free thiol groups, and chromomycin A_3 (CMA_3), which indicates a decreased presence of protamines. Hence, good quality spermatozoa would not fluoresce with CMA_3 or MMBr because they contain a normally protaminated chromatin that has been stabilized by disulphide cross-links. In all, 30 males were examined using CMA_3, while 21 were examined using MMBr; 200 spermatozoa were counted on each slide. All data were compared using the paired *t*-test.

Results: Spermatozoa recovered in the upper layer, when prepared using the swim-up technique, showed a significant ($P < 0.01$) increase in MMBr fluorescence compared with the fresh sample and with the spermatozoa in the sediment, indicating an increase in the number of spermatozoa without disulphide cross-links. The percentage of spermatozoa positive to the CMA_3 fluorochrome did not differ. When spermatozoa were prepared using the mini-Percoll technique, the number of spermatozoa recovered also showed a significant ($P < 0.01$) increase in MMBr fluorescence compared with the fresh sample. In contrast, there was a significant ($P < 0.01$) decrease in CMA_3-positive spermatozoa after mini-Percoll preparation compared with the fresh sample, indicating that the separation

method was able to provide more spermatozoa with normally protaminated chromatin in the preparation.

Conclusion: These results indicate that the populations of spermatozoa collected after swim-up and mini-Percoll preparation differ. Neither technique is able to eliminate spermatozoa with anomalies in the form of disulphide cross-links. In contrast, the mini-Percoll technique can distinguish, and enrich, the population of spermatozoa with a more condensed protaminated chromatin.

Reproductive endocrinology 03

Tuesday 24 June 1997
Hall C: Fintry Suite

16.45–17.00

O-137. Is there a difference in the function of granulosa cells in patients undergoing IVF with either GnRH agonist or GnRH antagonist

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Introduction: GnRH has a role in regulating gonadotrophin release. It has been shown that GnRH may also have a direct effect on the ovary because the addition of GnRH to a granulosa cell culture inhibits the production of progesterone and oestradiol. Specific GnRH receptors have been found to be present in rat and human granulosa cells, although the function of these receptors is unknown. Desensitization of the pituitary by GnRH agonist has become common practice in IVF, usually by a long protocol of 2–3 weeks. With the introduction of GnRH antagonists, which immediately block GnRH receptors, a much shorter period of exposure is needed of 3–6 days. The aim of this study was to evaluate the effect of the GnRH agonist Buserelin® and the GnRH antagonist Cetrorelix® on the function of granulosa cells cultured *in vitro* from IVF patients.

Materials and methods: Women aged <38 years seeking IVF or ICSI treatments were included in this study. They were prospectively randomized to have either Buserelin nasal spray (150 μg four times daily; group 1) from day 21 in the previous cycle and for 16 days before ovarian stimulation by 150 IU/day HMG, or Cetrorelix (group 2) which started with 150 IU HMG on cycle day 2 and 0.25 mg Cetrorelix from day 6 of the cycle. HCG was administered when the follicles were >17 mm in diameter. Oocytes were aspirated ~36 h later. Granulosa cells, separated and washed in EBSS from large follicles containing an ovum, were pooled. After 48 h of preincubation in Medium 199 (with 1% FBS added), the granulosa cells were cultured for 4 days in the above medium supplemented with either 0.5×10^{-5} M testosterone or 1 mM dibutyryl c-AMP with or without 1 IU/ml HCG, with a change of medium after 2 days. The progesterone and oestradiol concentrations in the culture medium were measured by an immunological assay, and cellular protein by a microprotein assay.

Results and conclusions: The granulosa cells from the women regulated by the GnRH antagonist showed a higher degree of steroidogenesis (two to three times) in all cultures (testosterone, HCG, c-AMP) than the granulosa cells obtained from the women regulated by GnRH agonist. This may be important for managing the luteal phase in IVF cycles.

17.00–17.15

O-138. Gonadotrophin surge-inhibiting factor inhibits GnRH-stimulated mitogen-activated protein kinase activation

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Introduction: GnRH released by the hypothalamus potentiates the pituitary response to its own action, i.e. the stimulation of LH release. This 'self-priming' phenomenon involves the de-novo synthesis of so-called 'self-priming associated proteins' (SPAP). Self-priming is antagonized by a putative ovarian hormone, gonadotrophin surge-inhibiting factor (GnSIF), which neutralizes the action of SPAP. A number of signal transduction pathways converge at the mitogen-activated protein kinase (MAPK) level. Because MAPK is also activated by GnRH, we investigated whether GnSIF interfered with GnRH-stimulated LH release upstream or downstream of MAPK. In addition, we investigated whether c-AMP was a mediator in this action of GnSIF.

Materials and methods: Mouse gonadotrophic αT3-1 cell cultures, or pituitary cells from 28 day old female Wistar rats, were seeded in six-well plates (400 000 cells/well) and incubated for ≥24 h in serum-free medium to reset activated MAPK levels (phosphorylated MAPK; MAPK-P) to baseline values. The cells were then incubated for 15 min with media containing 1 μM GnRH, highly purified bovine GnSIF preparations or GnRH with GnSIF added at 0, 5 and 10 min after the start of GnRH stimulation. In the homologous αT3-1 cell line, GnSIF was also replaced with the non-hydrolysable c-AMP analogue 8-Br-c-AMP (3 mM) to mimic c-AMP signalling. The cells were lysed in SDS–PAGE loading buffer, and the lysates fractionated on 8.5% SDS–polyacrylamide gels. MAPK activation was quantitated on Western blots by determining the ratio between the MAPK and MAPK-P (migrating 1–2 kDa higher as MAPK) protein band densities.

Results: αT3-1 cells showed a 3.5-fold increase in the MAPK-P/MAPK ratio when stimulated with GnRH. No stimulation was found with the GnSIF preparations, and no inhibitory effect on GnRH-stimulated MAPK activation was observed. When pituitary cells were incubated with GnSIF, no increase was seen in the MAPK-P/MAPK ratio. However, in contrast to the αT3-1 cells, GnSIF markedly decreased GnRH-stimulated