Summary answer: Corifollitropin alfa followed by hp-HMG does not increase ongoing pregnancy rates compared with rFSH in young poor responders. However, more supernumerary cryopreserved embryos are obtained.

What is known already: Poor ovarian response (POR) remains one of the main therapeutic challenges in women undergoing ovarian stimulation, given that very low live birth rates of 6% have been reported in this special group of infertile patients. Nevertheless, concerns have been raised that a degree of heterogeneity remains, as the prognostic effect of individual factors is still unclear, particularly for the young poor responder group. The rationale for conducting the current randomized trial lies to a previous pilot study demonstrating promising results with the administration of hp-HMG following corifollitropin alpha in women less than 40 years of age, fulfilling the "Bologna" criteria.

Study design, size, duration: This is a multicenter, phase III superiority randomized trial using a parallel two –arm design. The study included 152 patients <40 years old, fulfilling the "Bologna" criteria for POR from 1 tertiary referral center in Europe and 1 tertiary referral center in Asia, who underwent ovarian stimulation for ICSI from March 2013 to May 2016. Randomization sequence was performed using a computer generated randomization list, stratified by center, using 1:1 allocation.

Participants/materials, setting, methods: Eligible patients were randomized to either administration of 150 µg corifollitropin alfa followed by 300 IU hp-HMG (Group A) or to 300 IU of daily recombinant FSH (Group B) in a fixed GnRH antagonist protocol. The primary outcome was ongoing pregnancy rates (defined as presence of intrauterine gestational sac with an embryo with cardiac activity at 9-10 weeks of gestation). Secondary outcomes included clinical /biochemical pregnancy rates and number of oocytes retrieved cryopreservation rates.

Main results and the role of chance: Overall, 152 poor ovarian responders defined by the "Bologna" criteria were included in the study. Using an intention-to treat analysis the ongoing pregnancy rates did not differ significantly between Group A 11/77 (14.3%) and Group B 11/75 (14.7%), OR = 1.03 (0.4–2.5). Biochemical/clinical pregnancy rates and the number of oocytes retrieved were also comparable between the two groups. Nevertheless, more patients in the corifollitropin alfa group had cryopreserved embryos compared to recombinant FSH [14 (31.1%) versus 6 (15.8%), OR = 2.4 (1.04–5.5)]. Furthermore, Asian poor responders had significantly lower cancellation rates compared to European poor responders [2/64 (3.1%) versus 17/83 (20.4%), OR = 0.12 (0.03–0.5)]. This discrepancy could be explained by the fact that Asian women were of better prognosis than European patients, with significantly lower FSH [9.8(5.3) versus 11.5(5.4), p = 0.017] and significantly higher AMH [1.1(0.9) versus 0.4(0.3), p value <0.001].

Limitations, reasons for caution: Although we failed to identify differences between the two randomized arms, we cannot exclude that smaller differences might exist, which our study was underpowered to detect.

Wider implications of the findings: POR represents a challenge and although specific protocols may increase the number of cryopreserved embryos, no difference is observed in ongoing pregnancy rates. Our study, being one of the largest RCTs in Bologna poorresponders, highlights that baseline characteristics may play a crucial role in the clinical prognosis of this population.

Trial registration number: The EUDRACT number of the trial was 2013-000583-29 and the study was registered to clinicaltrials.gov (NCT01816321).

O-014 Letrozole step-up protocol: the effect of a novel superovualtion induction protocol to enhance pregnancy rate in a couple with unexplained infertility undergoing intrauterine insemination

A. Galal[1]

[1]*Alexandria, Egypt,*

Study question: investigate success of novel step-up protocol of letrozole as an attempt to achieve multifollicular development and to assess its effect on outcome of IUI cycles in couples with unexplained infertility.

Summary answer: letrozole step up protocol is potentially valid novel induction protocol, has privilege of similar pregnancy rate to standard HMG ovulation induction with lower cost

What is known already: Objective Empiric ovarian stimulation with clomiphene citrate, aromatase inhibitors (Letrozole) or exogenous gonadotropin is commonly combined with IUI in the treatment of couples with unexplained infertility. The successful use of the aromatase inhibitor,letrozole, for ovarian stimulation was documented when administered as a single dailydose or multiple daily fixed dose. To our knowledge,achieving consistent multifollicular development with letrozole, without the addition of FSH, has not been reported except in a retrospective trial.

Study design, size, duration: Design a prospective randomized controlled study.

Materials and Methods 100 couples with unexplained infertility undergoing IUI were randomized by computer number system into two groups.The sample size was calculated using Epi-Info 2002 software. Using a power of 80% to detect a significant difference at conception rate after IUI between the two groups = 19% with 5% precision and alpha error = 0.05, the sample size was 50 couples per group.

Participants/materials, setting, methods: ovulation stimulation by letrozole step up protocol from day 2, 3 of menstrual cycle starting by dose 2.5 mg increased daily by 2.5 mg for next 3 days.Group B, HMG ampoules 75 iu, tailred according to response,HCG when leading follicle 17 mm, IUI done 36 hours later.luteal support by vaginal progesterone, serum B HCG was measured after 14 days, clinical pregnancy by fetal heart pulsation 6–8 weeks

Main results and the role of chance: The step-up letrozole protocol was associated with multifollicular ovarian development with a mean of 1.5 ± 0.7 that was less than this detected with HMG 3.1 ± 1.0 however this did not affect so much clinical pregnancy rate that was statistically insignificant (16% in letrozole group versus 18% in HMG group) that may reflect the good quality of oocyte in addition to the good receptivity suggested by non significant difference in endometrial thickness. The cost of letrozole cycles was significantly lower than HMG group.

Limitations, reasons for caution: small scale

Wider implications of the findings: can be applied on a large number of patients

Trial registration number: ACTRN12614000024640

SELECTED ORAL COMMUNICATIONS

SESSION 04: THE ROLE OF GENES TESTIS STRUCTURE IN MALE INFERTILITY

Monday 3 July 2017 Room A 10:00–11:30

O-015 Blood-testis barrier organization in a prepubertal and peripubertal boys' cohort: correlation with Sertoli cell maturation, clinical puberty and testicular anatomopathology

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Study question: How do blood-testis barrier (BTB) proteins correlate with Sertoli cell maturation, testicular histopathological and clinical characteristics during transition from prepubertal to pubertal stages?

Summary answer: We observed that BTB proteins expression and organization were correlated to Sertoli cell maturation and to increasing age, in association with histopathological and clinical puberty

What is known already: The BTB consists of tight and gap junctions, which preserve paracrine interactions between Sertoli and germ cells as well as the

migration of differentiating germ cells towards the seminiferous lumen. Claudin 11 and connexin 43 are main proteins of tight and gap junctions, respectively. In connexin 43 knockout mice, Sertoli cells do not fully differentiate, implying thus that the BTB is essential for their maturation. In humans knowledge on the BTB is limited to adult tissue, where abnormal BTB formation was associated with impaired spermatogenesis. However, before puberty and during the pubertal transition, BTB establishment has not yet been described.

Study design, size, duration: The study was designed to assess the dynamic evolution of the BTB in a cohort of pre- and peripubertal boys. 49 patients, aged 0–15 years, who underwent a testicular biopsy to preserve their fertility before gonadotoxic treatment and had no previous history involving risks for infertility were selected. Correlations between the presence of BTB proteins, patient's age and Sertoli cell maturation were analyzed.

Participants/materials, setting, methods: Connexin 43 and claudin 11 immunostaining was performed to evaluate the BTB. A scoring system was used to assess their absence or disorganized-organized presence. Sertoli cell maturation was evidenced by Anti Mullerian hormone (AMH) immunohistochemistry. Tanner stages and the histological presence of haploid cells were recorded. AMH evolution, association between age and BTB, and correlation between AMH and BTB proteins were analyzed with linear regression, Fischer's test and Spearman correlation, respectively.

Main results and the role of chance: Connexin 43 and claudin 11 expressions increased significantly with age ($p = 0.04$ and $p \leq 0.01$, respectively). Connexin 43 was expressed in a disorganized state from the first year of age and its organized expression was only observed after 12 years of age, simultaneously with the onset of claudin 11 expression, the presence of haploid germ cells and the progression towards more advanced Tanner stages. AMH staining decreased significantly with age ($p \leq 0.01$), showing a progressive maturation of Sertoli cells. Moreover, we observed an inverse correlation between the expression of AMH and both connexin 43 ($p = 0.05$) and Claudin 11 ($p < 0.01$), indicating that Sertoli cell maturation is linked to the organization of the BTB. We showed for the first time, in a cohort of pre- and peripubertal boys, that the progression through puberty, demonstrated by Tanner stages and by testicular histological analysis, was simultaneous to the establishment of an organized BTB and Sertoli cell maturation. Further studies on the association between BTB components and onset of spermarche during the pubertal transition period are required to increase knowledge on differentiation of prepubertal testicular tissue and achieve in vitro maturation of immature testicular tissue.

Limitations, reasons for caution: Assessment of more BTB proteins may help to fully understand its establishment. The size of the population of peripubertal boys should be increased to study the correlation between germ cells at all stages of differentiation and the BTB and understand how alterations of the formation of BTB can affect spermatogenesis.

Wider implications of the findings: Since the knowledge on the human BTB in a pre-peripubertal cohort was lacking, our data provide a control population which can serve to assess in vitro maturation protocols for prepubertal testicular tissue. Furthermore, it may also be useful for in vivo applications as male contraception.

Trial registration number: not applicable.

O-016 Gradient system for testicular organoids generation – a novel system to model germ to somatic cell association in vitro

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Study question: Can germ and somatic testicular cells reorganize *in vitro* in a close to *in vivo* association if co-cultured in a three dimensional gradient system (3DGS)?

Summary answer: Germ and Sertoli rat cells reorganized in seminiferous-like structures when co-cultured in the 3DGS allowing the study of germ-to-somatic cell interactions *in vitro*.

What is known already: Germ cell proliferation and differentiation are delicate and complex processes governed by a broad network of factors and somatic cells. These signaling pathways and cell-to-cell interactions have been exhaustively studied, but a lot still remain unknown. Different approaches such as organ culture or *de novo* formation of seminiferous-like structures from primary testicular cells have been applied to investigate the mechanisms that govern germ cell fate decision into proliferation or differentiation. However, a more efficient and controlled model which recapitulate the germ-to-somatic cell associations is still need to study the germ cell niche *in vitro*.

Study design, size, duration: Primary testicular cells from 20 *dpp* rat were culture for 10 and 21 days using the 3DGS in basic culture condition. The effect of the treatment for 10 days with retinoic acid (RA) IL-1α, TNFα and RA inhibitors in germ cell maintenance and BTB organization was compared to the control culture conditions for the same period of time.

Participants/materials, setting, methods: For the gradient system setting, 3 concentric drops of Corning® Matrigel® diluted 1:1 with culture medium were sequentially applied on the bottom membrane surface of a hanging cell insert. The middle drop had a final cell concentration of 44 million cells/mL. DMEM-α supplemented with 1% pen/strep and 10% KnockOut serum replacement was used as basic culture medium. Evaluation of the results was done by bright-field microscopy and by confocal microscopy after whole-mount staining.

Main results and the role of chance: Sertoli and germ cells reassembled in spherical-tubular structures (STSs) showing similarities to seminiferous tubules organization. The characterization of STSs revealed that they are mainly formed by epithelized Sertoli cells. Moreover, the formation of a blood-testis barrier (BTB) *in vitro* was demonstrated by the detection of Zo-1 and occluding proteins between Sertoli cells and by the impermeability of the spherical-tubular structures to Evans Blue, a small molecule that cannot cross healthy BTB *in vivo*. Additionally, germ cells could be maintained for 21 days on the STSs. Furthermore, undifferentiated germ cells were observed to proliferate and formed cellular chains in a similar way as observed *in vivo*.

In order to validate the 3DGS to investigate signaling pathways and cell-to-cell interactions in the germ cell niche, we verify the role of retinoic acid (RA), IL-1α, TNFα and RA inhibitors in germ cell maintenance and BTB organization in vitro. RA treatment had a positive effect in germ cell maintenance compared with control conditions. Furthermore, IL-1α and TNFα were observed to impair the formation of testicular organoids and germ cell maintenance. Thus, we demonstrated our 3DGS to be a new model to explore germ cell niche *in vitro*.

Limitations, reasons for caution: The testicular organoids do not completely mimic testicular physiology yet. More specifically, progression in spermatogenesis was not observed in the basic culture conditions utilized mainly due to the lack of knowledge regarding the factors involved in germ cell differentiation.

Wider implications of the findings: The 3DGS constitutes a new method to generate testicular organoids representing a unique model of germ-to-somatic cell association *in vitro* with possible application to search for factors involved in the germ cell niche regulation. Moreover, the model might be applied to generate organoids and study organogenesis in other scientific fields.

Trial registration number: Not applicable.

O-017 In vitro re-assembly of human primary testicular cells into seminiferous cord-like structures

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Study question: Can enzymatically dispersed testicular cells from adult men re-organize into seminiferous cords *in vitro*?

Summary answer: Adult human testicular somatic cells re-assembled into testicular cord-like structures consisting of Sertoli and peritubular cells showing dynamic interactions.

What is known already: Attempts to induce spermatogenesis *in vitro* have a long lasting history with no success in human so far. Current evidence from animal studies suggests that an intact testicular somatic microenvironment is required to support germ cells. The capacity of testicular cell suspensions from adult prostate cancer patients to self-organize in spheroid testis-like units, albeit