"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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Document type : Communication à un colloque (Conference Paper)
**Summary answer:** Corffolitropin alfa followed by hCG-HMG does not increase ongoing pregnancy rates compared with FSH in young poor responders. However, more supernumerary cyropreserved 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 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 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 hCG-HMG following corffolitropin 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 corffolitropin alfa followed by 300 IU hCG-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, cryopreserved rates.

**Main results and the role of chance:** Overall, 152 poor ovarian responders fulfilled 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/cclinical pregnancy rates and the number of oocytes retrieved were also comparable between the two groups. Nevertheless, more patients in the corffolitropin alfa group had cryopreserved embryos compared to recombinant FSH [14 (31.1%) versus 6 (15.8%), OR = 2.4 (1.04–5.3)]. Furthermore, Asian poor responders had significantly lower cancellation rates compared to European poor responders [2/6 (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 0.40 (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 poor responders, highlights that baseline characteristics may play a crucial role in the clinical progression of this population.

**Trial registration number:** The SUDRACT number of the trial was 2013-000593-29 and the study was registered to clinicaltrials.gov (NCT01816321).

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**SELECTED ORAL COMMUNICATIONS**

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

**Monday 3 July 2017**

Room A 10:00–11:30

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**O-015 Blood-tests 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-tests 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 peripuberal 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 Muierian 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 = 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 = 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 = 0.005) 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 peripuberal 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 spermatogenesis 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 influence spermatogenesis. Wider implications of the findings: Since the knowledge on the human BTB in a pre-peripuberal 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.

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**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 recapitulates the germ-to-somatic cell associations is still needed to study the germ cell niche in vitro.

**Study design, size, duration:** Primary testicular cells from 20 dd pfl rats were cultured for 10 and 21 days using the 3DGS in basic culture condition. The effect of 10 days treatment with 10 days with retinoic acid (RA) IL-1a, 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 Carneby™ 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-a 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 epithelial Sertoli cells. Moreover, the formation of a blood-testis barrier (BTB) in vitro was demonstrated by the detection of ZO-1 and including proteins between Sertoli cell tubules by the permeability of the spermatid 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-1a, 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-1a 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.

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**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

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*Abstracts of the 33rd Annual Meeting of ESHRE, Geneva, Switzerland 2 to 5 July 2017*