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Pre-*in vitro* maturation (IVM) system using PACAP improves meiotic maturation and developmental competence for porcine oocytes

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Introduction: In porcine, meiotic competence is acquired progressively by the oocyte during follicular growth.Even though oocytes become able to resume meiosisin small follicle with a diameter of <3mm in diameter (SF), the oocytes is needed some important events to occur in the oocyte during late follicular growth, contributing to its full developmental competence. To improve capacitation of oocytefrom SF and inhibit spontaneous maturation, pre-in vitro maturation (IVM) system was developed for *in vitro* culturing cumulus-oocyte complexes (COCs). The purpose of this study is to establish the optimal phase and exogenous addition of pituitary adenylate cyclase-activating peptide (PACAP) on pre-IVM.

Materials and Methods: To establish the appropriate phase and concentrationof PACAP on pre-IVM, we assessed nuclear status according to culture duration (0h, 6h, 12h, 18h and 24h) and concentration (0, 500fM, 100pM, 10nM and 1uM). In the conventional IVM group, COCs were obtained from follicles ≤3mm in diameter (SF) and ≥3mm in diameter (MF) and subjected to IVM for 42h. In the pre-IVM group, COCs were only obtained from SF and matured with non treatment (Pre-SF(-)PACAP) and 1uM PACAP (Pre-SF(+)PACAP) for 60h including pre-IVM 18h. The each groups were compared in term of nuclear maturation, intracellular GSH and ROS levels and embryo developmental competence.

Results: The result of the nuclear stage assessment of the COCs from SF are as follow: Germinal vesicle (GV) stage of 0h (99.4%), 6h (96.8%), 12h (87.2%), 18h (84.1%)and 24h (64.9%). The rate of GV between 18h and 24h groups was statistically significant difference (P<0.05). PACAP was treated on pre-IVM as mentioned previously. After 18h, we evaluated the rate of GV stage. The 10uM group showed a significantly (P<0.05) the highest rate on meiotic arrest of COCs: GV stage of control (60.5%), 500fM (64.%),100pM (74.4%), 10nM (69.9%) and 1uM (82.1%). The result of the nuclear stageassessment of groups for Metaphase Ⅱ are as followed: MF (81.7%), SF (68.2%), Pre-SF(-)PACAP (81.7%) and Pre-SF(+)PACAP (91.7%). The Pre-SF(+)PACAP group was significantly the highest Metaphase Ⅱ rate (P<0.05). MF and Pre-SF(+)PACAP groups and SF and Pre-SF(-)PACAP groups in GSH levels showed statistically significant difference (P<0.05). After PA, the cleavage rateswere significantly (P<0.05) higher in Pre-SF(+)PACAP group than the other groups.

Conclusions: These results indicated that pre-IVM system using PACAP is able to delay oocyte meiotic maturation during pre-IVM phase and improve meiotic maturation and developmental competence.

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Treatment of GDF8 on Oocyte Maturation activated SMAD2/3 signaling and improved subsequent invitro embryonic development

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Introduction: Growthdifferentiation factor8 (GDF8) is a member of the transforming growth factor-β that has been identified as a strong physiological regulator. SB431542 (SB) is a specific inhibitor of transforming growth factor-beta superfamily type I activin receptor-like kinase (ALK) receptors. The purpose of this study is theeffects of GDF8 and SB on porcine oocytes *in vitro* maturation (IVM) and subsequent embryonic development after parthenogenetic activation (PA).

Materials and Methods: We were investigated the effect of GDF8 and SB treatment during IVM on nuclear maturation, intracellular glutathione (GSH), reactive oxygen species (ROS) levels, analyzed specific gene transcription and translation levels in cumulus cells after IVM, and embryonic development and transcription pattern after PA. Data were analyzed by on way ANOVA. The 1.318 ng/mL of GDF8 and 5ng/mL of SB were added during IVM followed experiment design as control, SB, SB+GDF8, and GDF8 treatment groups.

Results: After 44 h of IVM, GDF8 group (90.4%) showed significantly increased nuclear maturation than other groups (85.4%, 78.9%, 85.4% and 90.4%, respectively), and SB group showed significantly lower maturation than control (p <0.05). The GDF8 group showed significantly (P<0.05) decreased intracellular ROS and increased GSH levels compared with other groups. SB+GBF8 group showed significantly better cytoplasmic maturation than SB group. The GDF8 group showed highly increased *PCNA* and *Nrf2* and cumulus expansion factors *COX-2*, *Has2*, *Ptx3* and *TNFAIP6* mRNA transcription level in cumulus cells after IVM. In protein expression level, GDF8 group showed significantly increased phosphorylated SMAD 2/3 per SMAD 2/3 ratio than control(p

<0.05). In PA embryonic development, GDF8 group showed a significantly ($p<0.05$) higher blastocyst (BL) formation rate and total cell numbers compared with other groups (47.9% and 74.0, 37.2% and 62.9, 46.4% and 71.0, and 58.7%and 83.8 respectively). In the assessment of gene expression pattern in Pa BL, the developmental competence marker *PCNA* and *POU5F1*, and anti-apoptosisindicator *Bcl-2* transcription levels were significantly increased in GDF8 group when compared with control ($p <0.05$).

Conclusions: In conclusion, treatment of GDF8 during IVM significantly improved the PA embryo developmental competence and effected on transcription pattern, and redeemed developmental potential from SB inhibition by increasing P-SMAD2/3 ratio.

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Enhancedinterferon-mediated immune response in olive flounder (*Paralichthys olivaceus*) by DDx41- an immuno-adjuvant

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Introduction: DDx41, a cytoplasmic DNAsensor, belongs in the family of RNA and DNA helicases which possessesDExD/H-box domain [1]. Functioning as an initial sensor for cytoplasmic DNAs,DDx41 has been reported to be involved in the activation of type I interferon(IFN) immune response in olive flounder [2]. IFNs initiate intracellularantimicrobial response which then influence the development of both innate andadaptive immune responses [3]. IFN is considered to be one of the mostessential components of the immune system because of its ability in suppressingviral replication; hence, making it the main emphasis of most vaccinedevelopment studies in generating a more efficacious vaccine. We constructedtwo DNA vaccines in this study, one containing the gene DDx41 (pEF-D) and theother a glycoprotein (G)-based DNA vaccine-incorporated DDx41. Here, weelucidated on the ability of DDx41 as an adjuvant for a G-based DNA vaccine.

Materials and Methods: Four tanks containing oliveflounders (± 5.2 g) were reared at 14℃, each tank had 30 fish in it. The fishwere intramuscularly immunized with 1 ug of the plasmid construct (empty vectorpEF-A, adjuvant pEF-D, and vaccine-adjuvant pEF-GD) diluted in 100 uL PBS while, 100 uL PBS were injected into one group this served as the negativecontrol set-up. At day 1, 3, and 14 post-vaccination three fish from eachexperimental group were sampled, kidney and spleen were collected. RNA wasextracted from the collected organs and two micrograms of total RNA were used for the synthesis of cDNA. The cDNA samples were diluted 10 times withRNAse-free water, these were used as template for qPCR. $\triangle\triangle$Ct method wasutilized to quantify the fold changes of the immune gene transcripts in each ofthe samples. The expression levels of the target genes were normalized to theexpression level of β-actin and were expressed as the fold change relative tothe average level in the PBS group which is regarded as 1. Fifteen dayspost-vaccination, the fish were infected with 100 uL of 1×10^6 virus/mL(viral hemorrhagic septicemia virus, VHSV). The number of deaths were recordeduntil ten days post-infection.

Results: To validate the fact that DDx41 can initiatetranscription of IFN-related genes, several genes that are known to beactivated in the innate immune response mechanism of fish were investigated.After intramuscular injection of the constructs, these genes (IFN-1, IFN-g,IRF-3, and ISG-15) showed mRNA expression in both kidney and spleen fromdifferent sampling days. In order to show that only type I interferon and noother types of interferon was induced by the adjuvant construct, pEF-D,expression of IFN-γ was also assessed. Results showed strong expression ofIFN-1 in both kidney and spleen at day 1 post-vaccination, the opposite is truein the case of IFN-γ as there was no significant increase in the expression inboth organs from any sampling days. Thisresult verifies that our construct, pEF-D can elicit transcription of type Iinterferon, which concurs with the previous studies about DDx41 inmammals. Cytosolic sensors like DDx41triggers downstream signaling that prompts the IFN-1 production pathway whichonly happens after phosphorylation of interferon-regulatory factors (IRFs) suchas IRF-1, -3 and -7. The expression of IRF-3 was strongly upregulated in thekidney and a minimal increase in the spleen at day 3 post-vaccination wasobserved. Once IFN-1 was activated, transcription of IFN-stimulated genes(ISGs) follows after. In this study, ISG-15 expression showed increase at day 1post-vaccination in the kidney but in the spleen up-regulation was observed atday 3 post-vaccination. Comparison between the pEF-D fish group with the pEF-GDfish group, results showed that fish vaccinated with pEF-GD exhibited severalfold higher transcription of IFN-1 and IRF-3 in kidney and spleen at day 1 postvaccination, except for ISG-15 wherein the expression was up-regulated at day 3post vaccination in spleen. The efficacy of the vaccine-adjuvant constructcould be attested by the inhibition of virus replication in virus-infectedfish, relative percent survival (RPS) in pEF-D treated fish is 72.3% whilepEF-GD treated fish is 100% protected from VHSV infection.

Conclusions: Basedon the results, apparent adjuvanticity of