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*Reproduction, Fertility and Development*

### Supplementary Material

#### **Sperm functionality is differentially regulated by porcine oviductal extracellular vesicles from the distinct phases of the estrous cycle**

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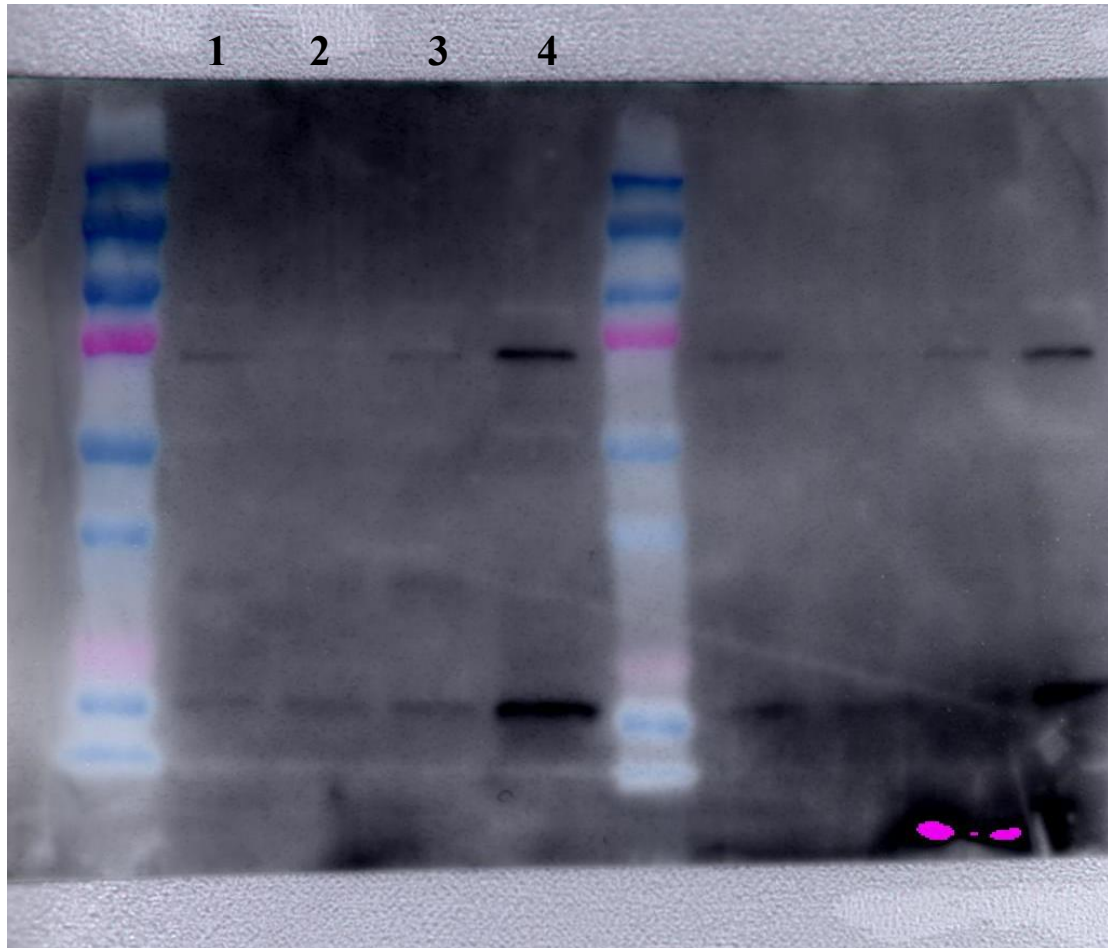
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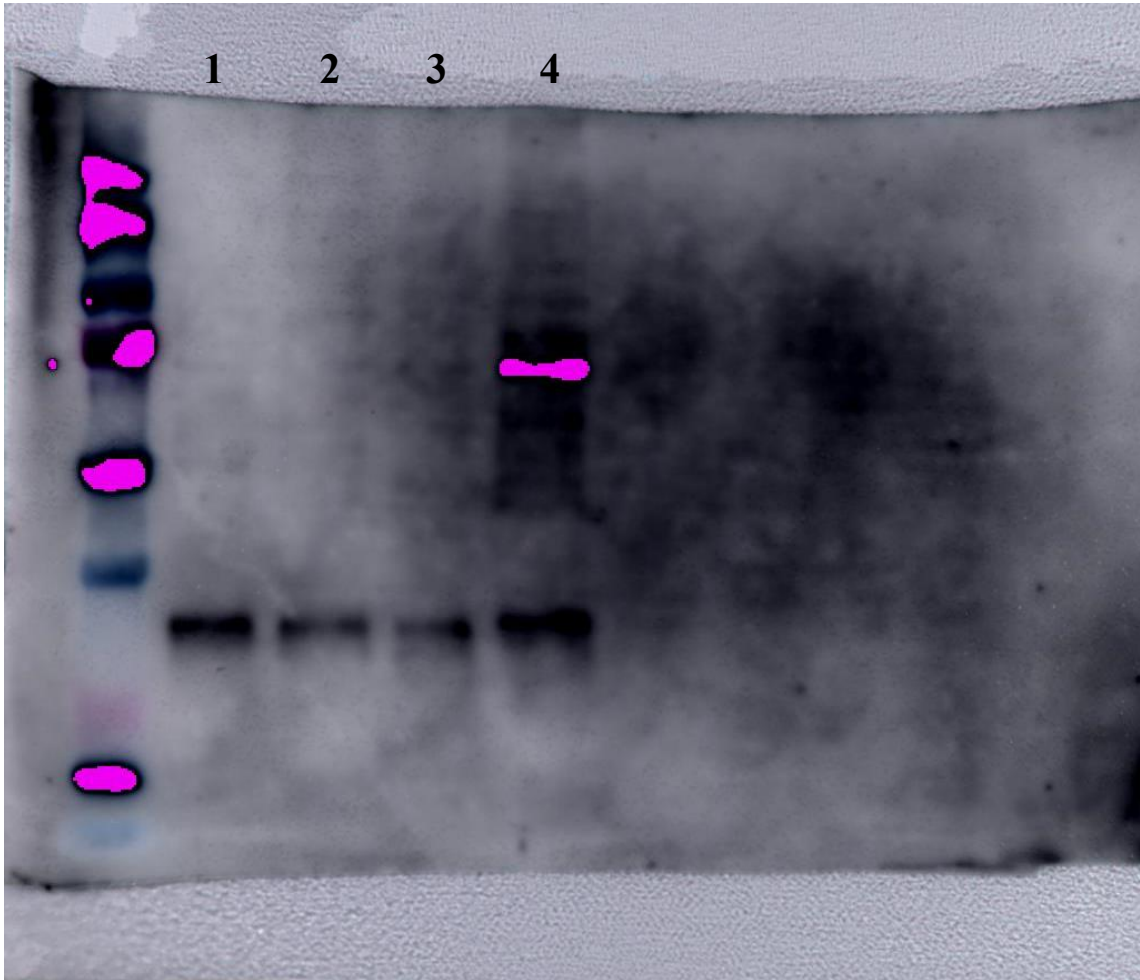
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## SUPPLEMENTARY MATERIAL

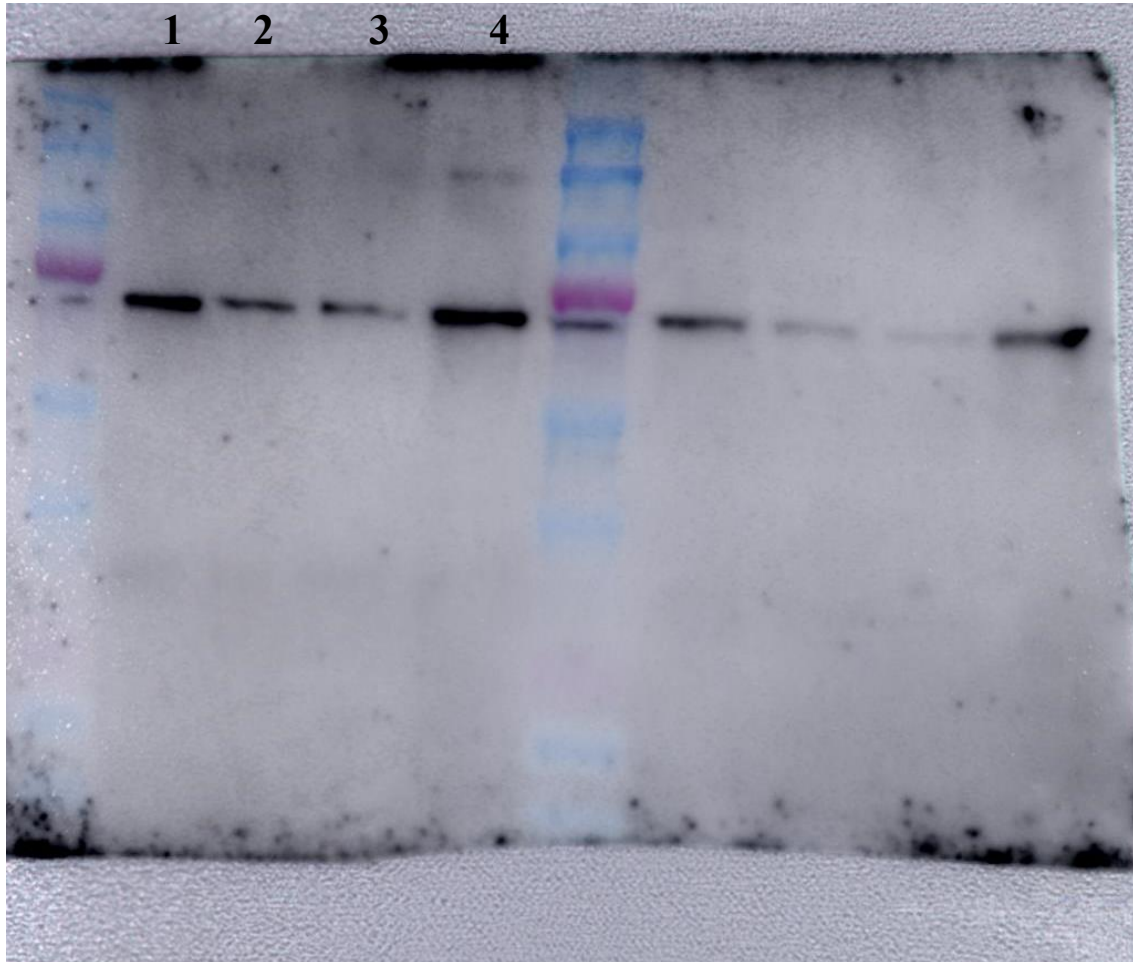
**Supplementary Figure S1.** Full blot of CD63 of poEVs of distinct phases of the estrous cycle. Lane 1: Late Follicular; Lane 2: Early Luteal; Lane 3: Mid Luteal; Lane 4: Late Luteal. Blocked with skimmed milk at 5% in T-TBS, Primary Antibody: anti-CD63 1:100 in T-TBS at 5% of skimmed milk, Secondary Antibody: HRP conjugated goat anti-mouse IgG 1:4,000 in T-TBS at 5% of skimmed milk.



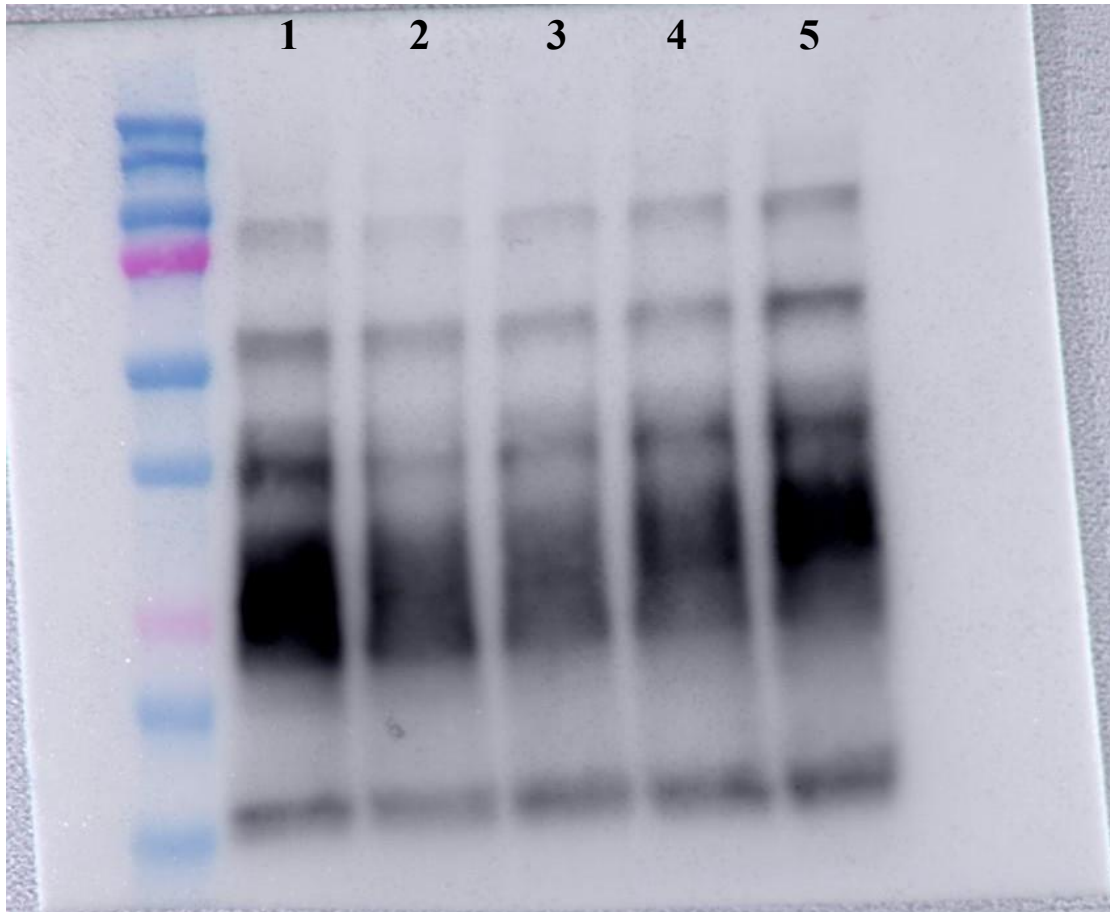
**Supplementary Figure S2.** Full blot of CD81 of poEVs of distinct phases of the estrous cycle. From the left; Lane 1: Late Follicular; Lane 2: Early Luteal; Lane 3: Mid Luteal; Lane 4: Late Luteal. Blocked with skimmed milk at 5% in T-TBS, Primary Antibody: anti-CD81 1:500n T-TBS at 5% of skimmed milk, Secondary Antibody: HRP conjugated goat anti-mouse IgG 1:10,000 in T-TBS at 5% of skimmed milk. The lanes were repeated twice.



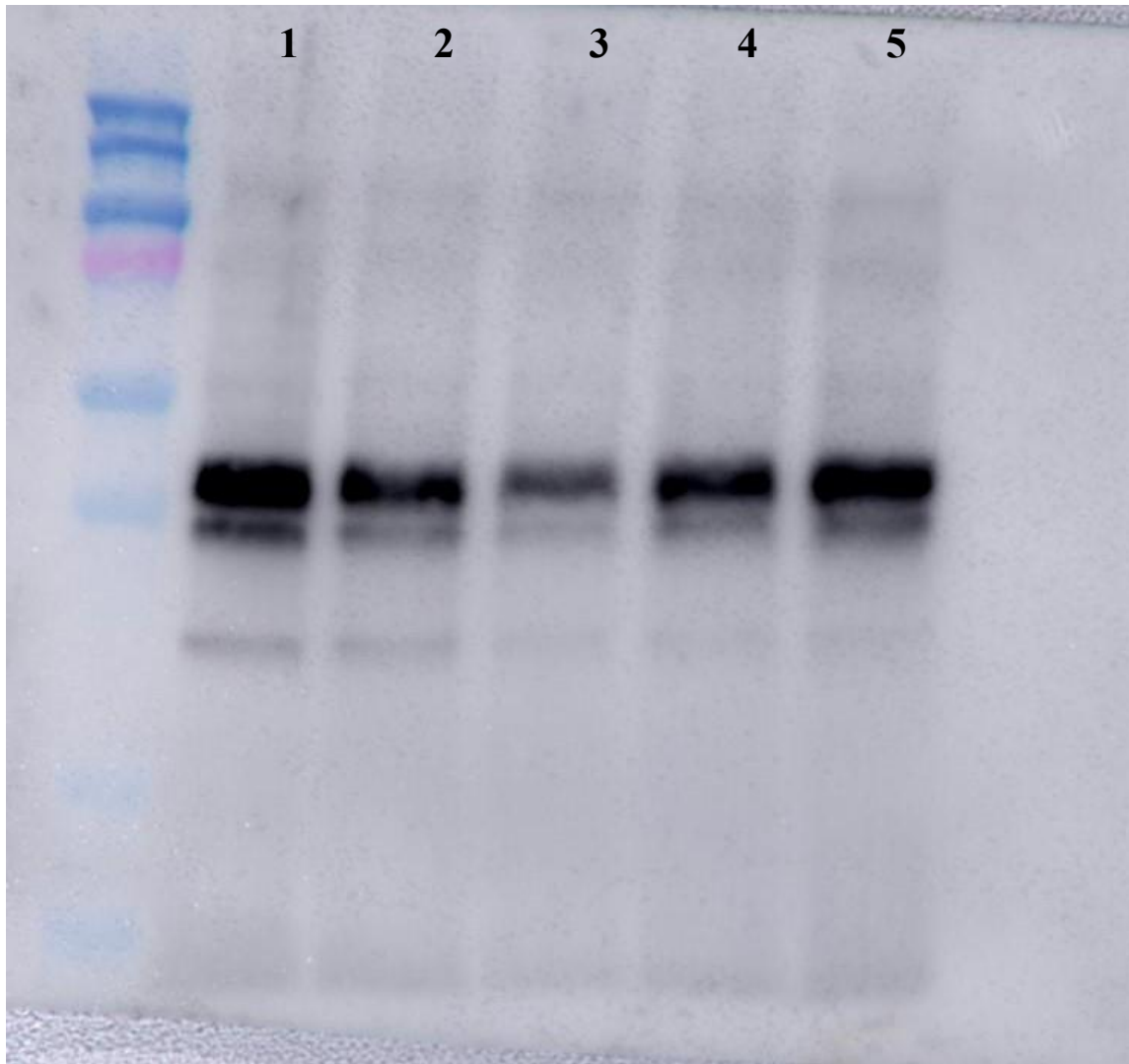
**Supplementary Figure S3.** Full blot of HSP70 of poEVs of distinct phases of the estrous cycle. From the left; Lane 1: Late Follicular phase; Lane 2: Early Luteal phase; Lane 3: Mid Luteal phase; Lane 4: Late Luteal phase. Blocked with skimmed milk at 5% in T-TBS, Primary Antibody: anti-HSP70 1:1000 in T-TBS at 5% of skimmed milk, Secondary Antibody: HRP conjugated goat anti-mouse IgG 1:10,000 in T-TBS at 5% of skimmed milk. The lanes were repeated twice.



**Supplementary Figure S4.** Full blot of protein kinase A of porcine sperm incubated with poEVs of distinct phases of the estrous cycle in TALP medium for 2 h. Lane 1: Control (Only spermatozoa) Lane 2: Spermatozoa plus poEVs of Late Follicular phase; Lane 3: Spermatozoa plus poEVs of Early Luteal phase; Lane 4: Spermatozoa plus poEVs of Mid Luteal phase; Lane 5: Spermatozoa plus poEVs of Late Luteal phase. Blocked with skimmed milk at 5% in T-TBS, Primary antibody: anti-pKA 1:10,000 in T-TBS at 5% of skimmed milk; Secondary Antibody: HRP conjugated goat anti-rabbit IgG 1:10,000 in T-TBS at 5% of skimmed milk.



**Supplementary Figure S5.** Full blot of phospho-tyrosine of porcine sperm incubated with poEVs of distinct phases of the estrous cycle in TALP medium for 2 h. Lane 1: Control (Only spermatozoa) Lane 2: Spermatozoa plus poEVs of Late Follicular phase; Lane 3: Spermatozoa plus poEVs of Early Luteal phase; Lane 4: Spermatozoa plus poEVs of Mid Luteal; Lane 5: Spermatozoa plus poEVs of Late Luteal phase. Blocked with BSA at 5% in T-PBS; Primary Antibody: anti-PY 1:10,000 in T-PBS at 5% of BSA; Secondary Antibody: HRP conjugated goat anti-mouse IgG 1:10,000 in T-PBS at 5% of BSA.



**Supplementary Figure S6.** Full blot of  $\beta$ -tubulin of porcine sperm incubated with poEVs of distinct phases of the estrous cycle in TALP medium for 2 h. Lane 1: Control (Only spermatozoa) Lane 2: Spermatozoa plus poEVs of Late Follicular phase; Lane 3: Spermatozoa plus poEVs of Early Luteal phase; Lane 4: Spermatozoa plus poEVs of Mid Luteal phase; Lane 5: Spermatozoa plus poEVs of Late Luteal phase. Blocked with BSA at 5% in T-TBS, Primary Antibody: anti- $\beta$ -tubulin 1:5,000 in T-TBS at 1% of skimmed milk, Secondary Antibody: HRP conjugated goat anti-mouse IgG 1:10,000 in T-TBS at 1% of skimmed milk.

