

# INFLUENCE OF THE EMBRYO STAGE, DEVELOPMENT KINETICS AND RECIPIENT SYNCHRONIZATION ON PREGNANCY RATES ON OPU-IVF EMBRYOS

## SUMMARY

Considering the existence of variation in physiology of *in vitro* produced embryos compared with *in vivo* and the possibility to evaluate developmental kinetics, it is reasonable to assume that variation in stage and time required to achieve specific stages may interfere on pregnancy. Here we compare pregnancy rates obtained from different stages of development. Discussion has focus on recipient synchronization. Data was produced by commercial IVF program based in São Paulo State – Brazil. Briefly, immature cumulus-oocyte complexes (COCs) were aspirated from donor cows by OPU, matured in TCM199 + 10%FCS + 0.5 $\mu$ g FSH/mL + 50 $\mu$ g LH/mL + 1 $\mu$ g estradiol/mL, for 24h at 38.5°C, 5% CO<sub>2</sub> in air. Live spermatozoa was obtained by centrifugation in Percoll gradients (45 and 90%) and cultured with COCs at 2 million of sperm/mL concentration in TALP medium + 10 $\mu$ g heparin/mL. After 10 to 18 hours incubation, zygotes carrying cumulus cells were transferred to recipients. Overall, 1,841 OPU sessions were done in 566 animals allowing the recovery of 19,659 viable oocytes, resulting an average of 3.87 transferable embryos per session (n=7,116). At day 7 of culture, 3281 embryos were classified at the specific developmental stage before being transferred, resulting in a pregnancy rate of 32.11%. Morula (Mo), initial blastocyst (Bi), Blastocyst (Bl), expanded blastocyst (Bx) and ecloded blastocyst (Be) presented respectively 16.16%(±0.87)<sup>d</sup>, 24.58%(±0.87)<sup>c</sup>, 31.41%(±0.83)<sup>b</sup>, 39.37%(±0.78)<sup>a</sup> of pregnancy rates. Concerning to the synchronization of the recipient, D5, D6, D7, D8, D9 (days after heat) presented respectively 15.48%(±0.92)<sup>c</sup>, 27.67%(±0.85)<sup>b</sup>, 38.01%(±0.79)<sup>a</sup>, 38.01%(±0.79)<sup>a</sup>, 39.78%(±0.79)<sup>a</sup> of pregnancy. These results, in one hand, suggest higher pregnancy potential for embryos that achieve blastocyst stage first. In other hand, best recipient synchronization was at days 7,8 and 9 after heat, suggesting that these embryos should be transferred in recipients latter in the estrous cycle.

## MATERIAL AND METHODS

### Follicular Aspiration

- \* Ultrasound Aloka SSD 500 - 5 Mhz
- \* Needle 18 G - 55 cm - 68mm Hg
- \* Medium OPU (PBS + 10 UI Heparin + 1% FBS + Antibiotic)
- \* Selection and transport (TCM 199 + Heparin + 10% FBS) - 34°C

### In vitro Maturation

- \* Evaluation of the oocyte quality (cumulus and cytoplasm)
- \* IVM (TCM199 + 10%FBS + 0.5 $\mu$ gFSH/ml + 5 $\mu$ gLH/ml + 1 $\mu$ g estradiol/ml)
- \* 24 hours - 5% CO<sub>2</sub> in air - 38.5°C

### In vitro Fertilization

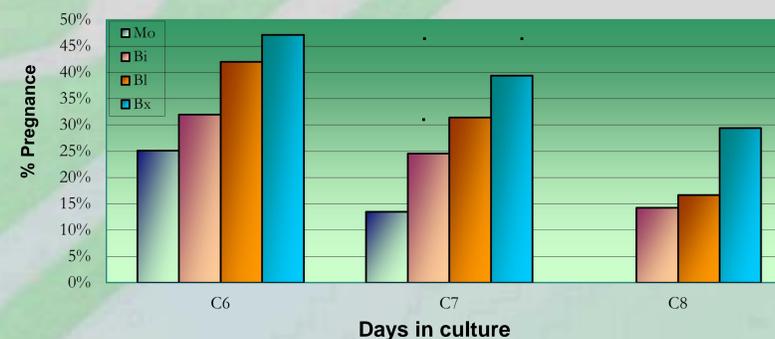
- \* Frozen-thawed semen
- \* Motile sperm cells - Percoll gradients (45-90%)
- \* Sperm concentration (2 x 10<sup>6</sup>cells/ml)
- \* IVF (TALP + BSA + PHE + 10 $\mu$ g/ml heparin)
- \* 5% CO<sub>2</sub> in air - 38.5°C - 12 hours

### In vitro Culture

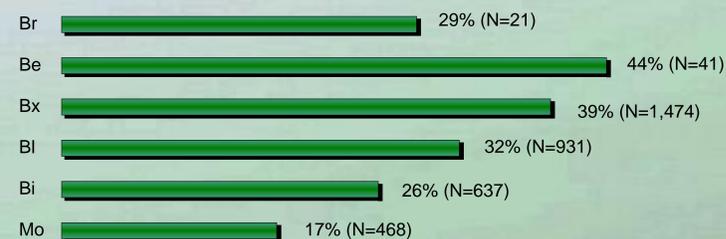
- \* Medium CR2 + 10% FBS with cumulus cells
- \* Feeding (D3)
- \* 5% CO<sub>2</sub> in air - 38,5°C - days 6-7
- \* Embryos transfer (fresh)

## RESULTS

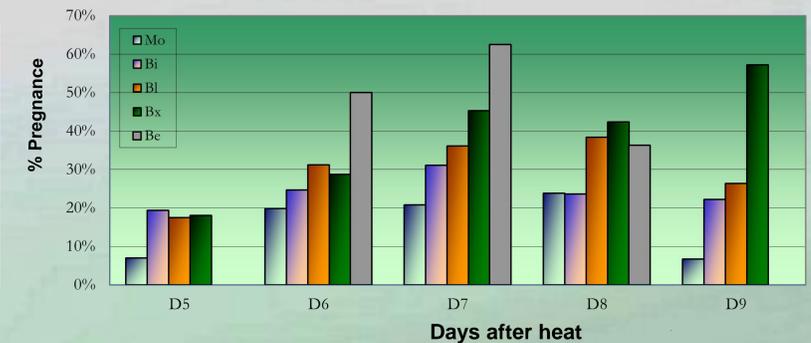
Influence of culture time on pregnancy



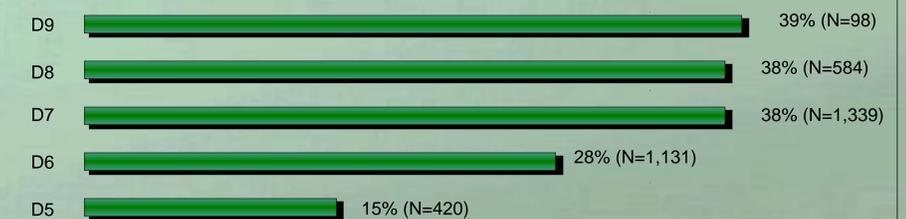
Influence of development stage on pregnancy



Influence of development stage and recipient synchronization on pregnancy



Influence of recipient synchronization on pregnancy



## CONCLUSION

- Higher pregnancy potential for embryos that achieve blastocyst stage first
- Best recipient synchronization was at days 7, 8 and 9 after heat
- Faster embryos in recipients later in estrous cycle provides better pregnancy rates