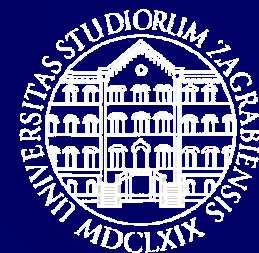# FOLLICULAR DYNAMICS AND IN VITRO EMBRYO PRODUCTION AFTER REPEATED SUPEROVULATION WITH FSH-P IN SIMMENTAL HEIFERS

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Ovum Pick-Up (OPU)

- Performed repeadly and safely during all reproductive stages in cattle

Stimulated (PMSG, FSH, IGF, BST)

or

Cycling donors

AIM OF THE STUDY:

- To compare the yields, morphological quality and developmental competence of oocyte-cumulus complexes collected from FSH-stimulated Simmental heifers in an initial and repeated superovulation and subsequent OPU sessions.

MATERIAL and METHODS

- 6 Simmental heifers aged 14-18 months
- $PGF_2\alpha$ synchronized
- Superovulation: pFSH a.m./p.m. during two days, starting on day 9 of the cycle

MATERIAL and METHODS
Reapeted stimulation of donor Simmental heifers for OPU/IVF (n=6):
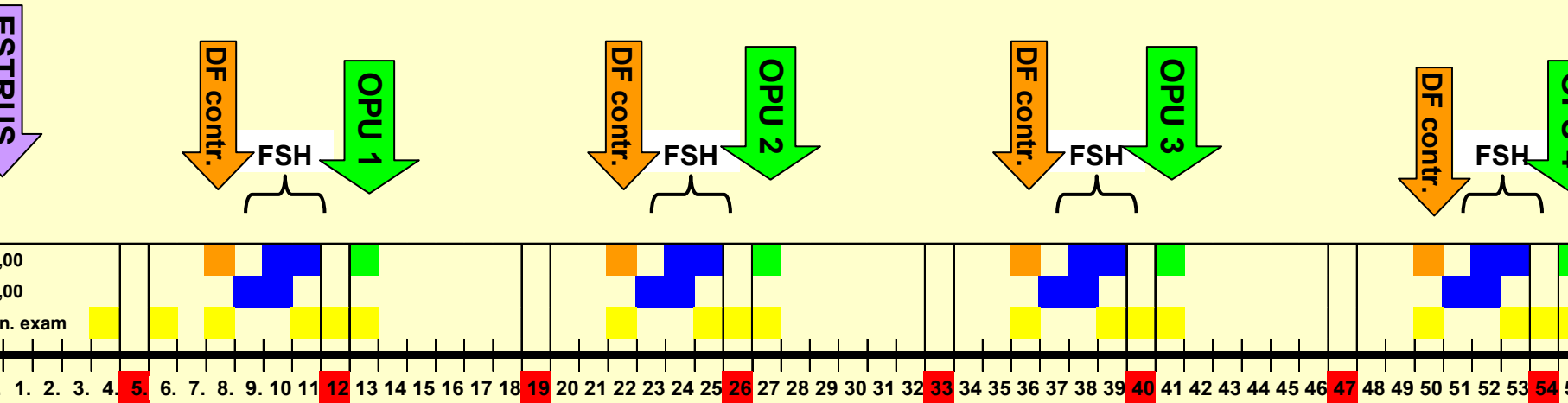
MATERIAL and METHODS

We registered:

- DF presence before superovulation
- Follicular dynamics 12, 24 and 48 h after FSH
- Follicle categories: 2-5 mm, 6-9 mm, 10-14 mm and >15 mm diameter
- Aspiration rate and quality of retrieved oocytes
- IVM/IVF/IVC rates

MATERIAL and METHODS
OPU Equipment

- ultrasound scanner with sector 5/7.5 MHz transducer (Pie Medical, the Netherlands)
- needle guidance system
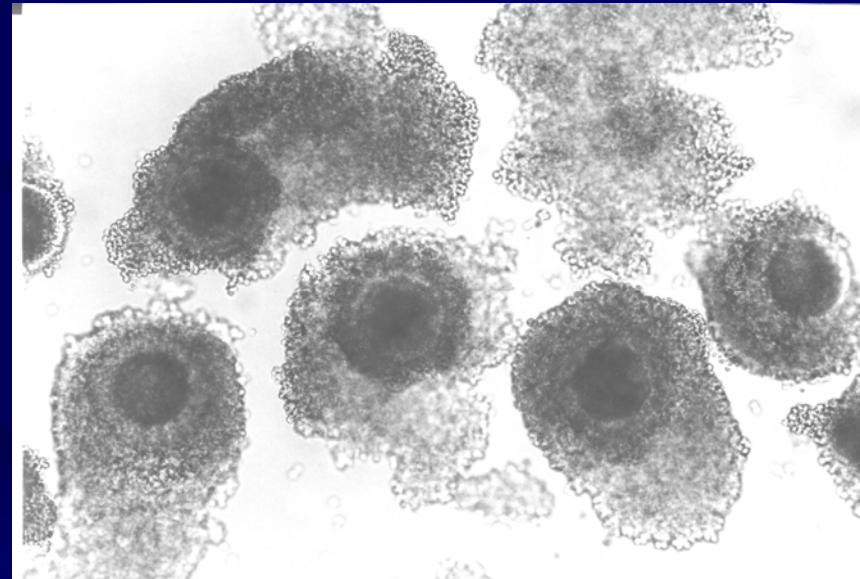- aspiration pump

MATERIAL and METHODS
Categorization of retrieved oocytes:

For IVM/IVF/IVC:

- Completely or partially surrounded with compact cumulus investment, even granulated ooplasm (G1+G2);

Out:

- Incomplete cumulus investment, unevenly granulated ooplasm (G3);

- "Nude" oocytes (G4),
  degenerated ooplasm;

- Expanded oocytes.

MATERIAL and METHODS
IVM/IVF/IVC

- **IVM:** TCM 199 with 10% FCS, FSH/LH, estradiol and cisteamin
  Incubation: 24^h at 39^oC and 5% CO_2

- **IVF:** bull semen prepared on discoutinous Percoll® gradient
  Final concentration for IVF: 10^6 sperms/ml

- **Culture in vitro:**
  SOFaaBSA at 39^oC
  5% CO_2, 7% O_2 i 88% N_2
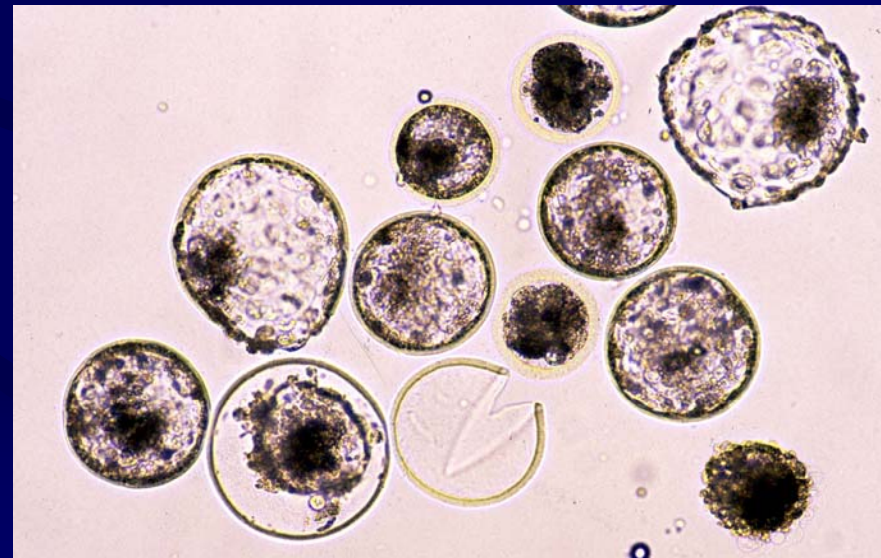
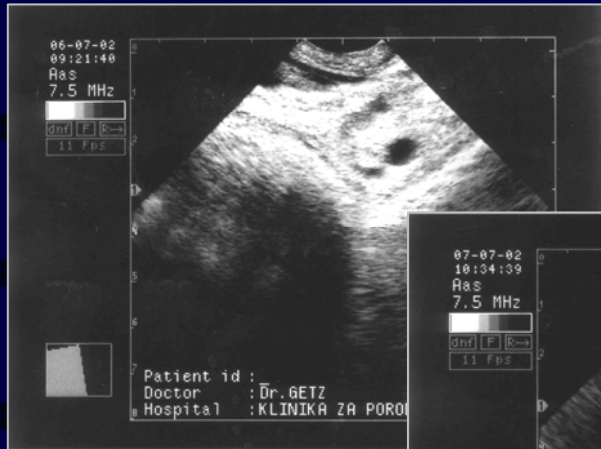# Evaluation of developmental competence of SO and OPU derived embryos

(according to IETS standards):

- Day 2 - No of cleaved embryos (fert. rate)
- Day 7 - No of morulas (M) and blastocysts (Bl)
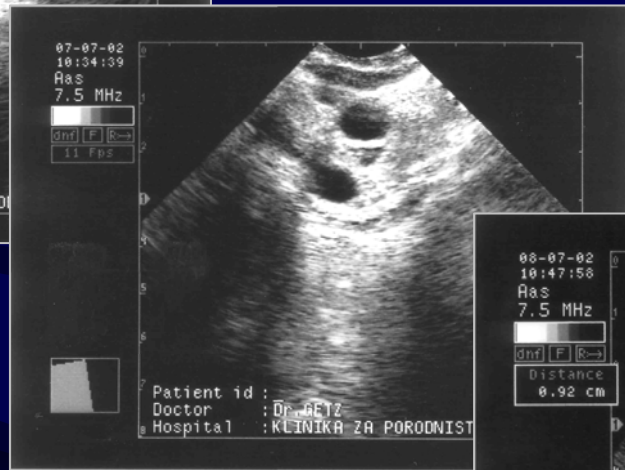- Day 11 - No of hatched blastocysts (hBl)

RESULTS

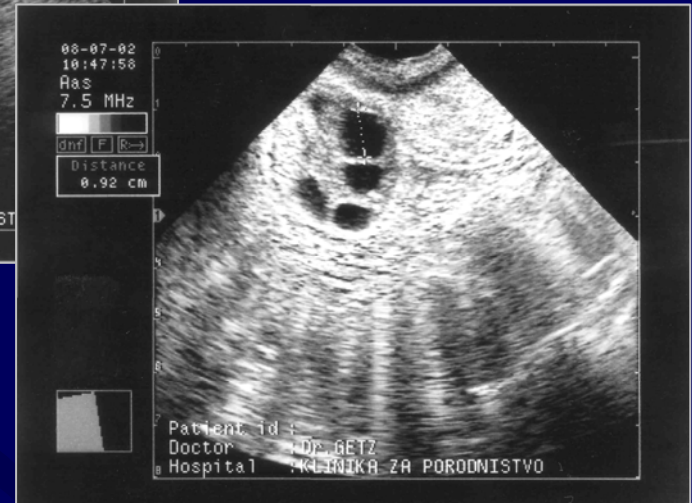## Follicular dynamics 12, 24 and 48 hours after reapeted FSH treatment

The echogram of the ovary

12 h after FSH

24 h after FSH

48 h after FSH

Figure 1: The mean number of different follicles categories 12, 24 and 48 hours after the repeated FSH treatments (OPU 1, OPU 2, OPU 3 and OPU 4).
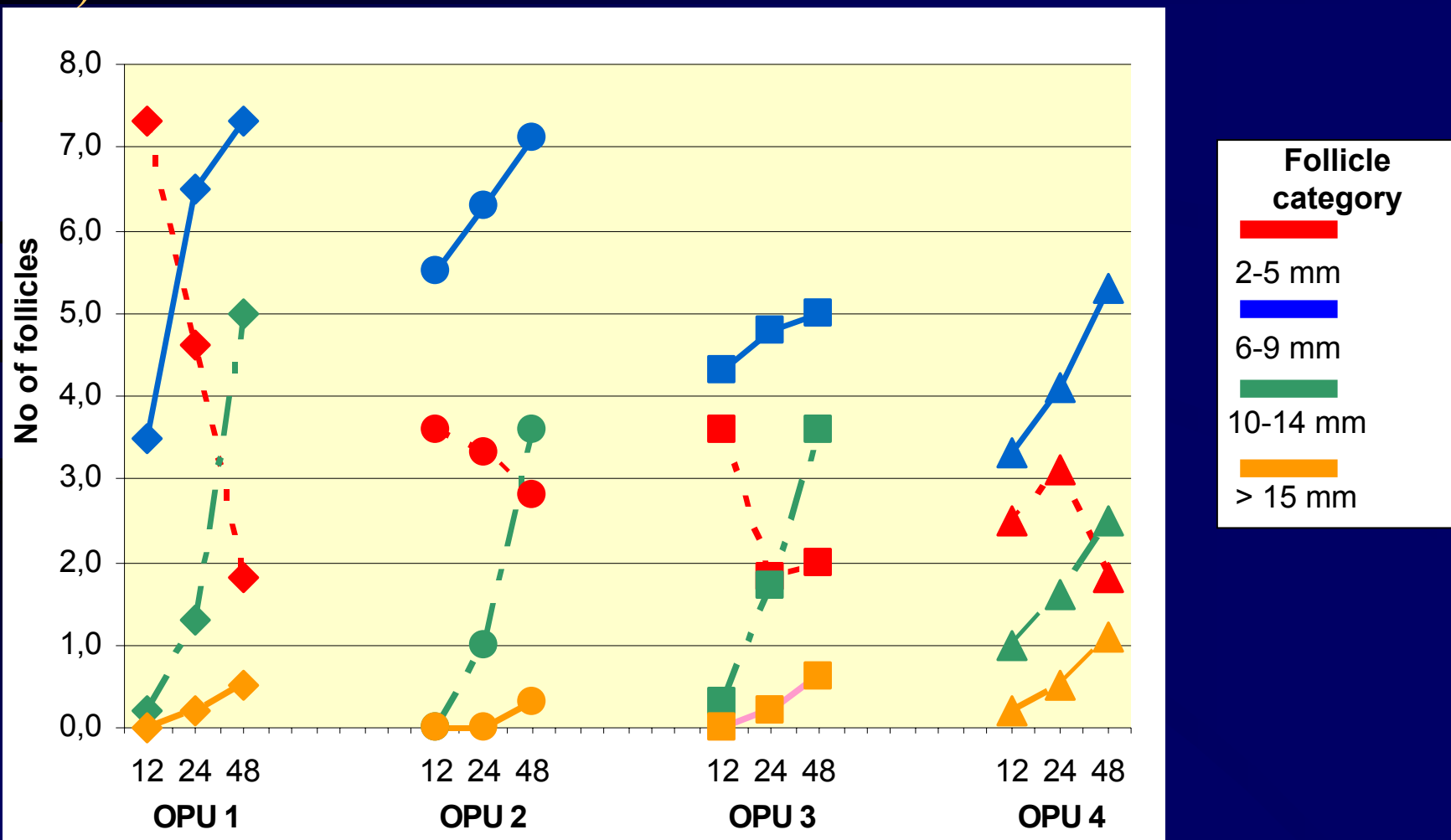
Table 1: The mean number (± SEM) of follicles 12, 24 and 48 hours after the last FSH injection in heifers with (DF+) and without (DF-) registered DF before the beginning of superovulation.

No.of follicles 12, 24 and 48 hours after FSH	12 h after FSH		24 h after FSH		48 h after FSH	
	DF+ (n = 10)	DF- (n = 14)	DF+ (n = 10)	DF - (n = 14)	DF+ (n = 10)	DF- (n = 14)
2 to 5mm	5.2 ± 1.1	3.9 ± 0.8	4.3 ± 0.9	2.5 ± 0.5	2.4 ± 0.5	1.9 ± 0.5
6 to 9mm	5.0 ± 0.7	3.9 ± 0.8	6.4 ± 0.7	4.7 ± 0.6	7.6 ± 1.0	5.2 ± 0.8
10 to 14mm	0.5 ± 0.3	0.2 ± 0.1	1.2 ± 0.4	1.6 ± 0.3	3.4 ± 0.6	3.9 ± 0.5
≥ 15 mm	0.1 ± 0.1	0.0 ± 0.0	0.4 ± 0.2	0.1 ± 0.1	0.8 ± 0.5	0.6 ± 0.2

* P<0,05

Table 2: The mean number (± SEM) of COCs collected after FSH ovarian stimulation and the mean number (±SEM) of different oocytes categories after the repeated FSH treatments (OPU 1, OPU 2, OPU 3 and OPU 4):

	COCs collected	G1 + G2	G3	G4
OPU 1 (n=6)	11.5 ± 1.7	9.5 ± 2.5	1.0 ± 0.6	0
OPU 2 (n=6)	9.1 ± 1.4	5.5 ± 1.0	1.8 ± 0.5	0.7 ± 0.3
OPU 3) (n=6)	6.3 ± 1.5	4.2 ± 1.0	0.8 ± 0.5	0.2 ± 0.2
OPU 4) (n=6)	7.8 ± 1.7	3.5 ± 1.3	1.8 ± 1.3	0.2 ± 0.2
Total	8.70 ± 0.91	5.67 ± 0.99	1.37 ± 0.38	0.25 ± 0.11

* P<0,05

Figure 2: Cleavage rate on D2, morulas/blastocysts rate on D7 and hatched blastocysts rate on D10 after four consecutive superovulatory treatments.
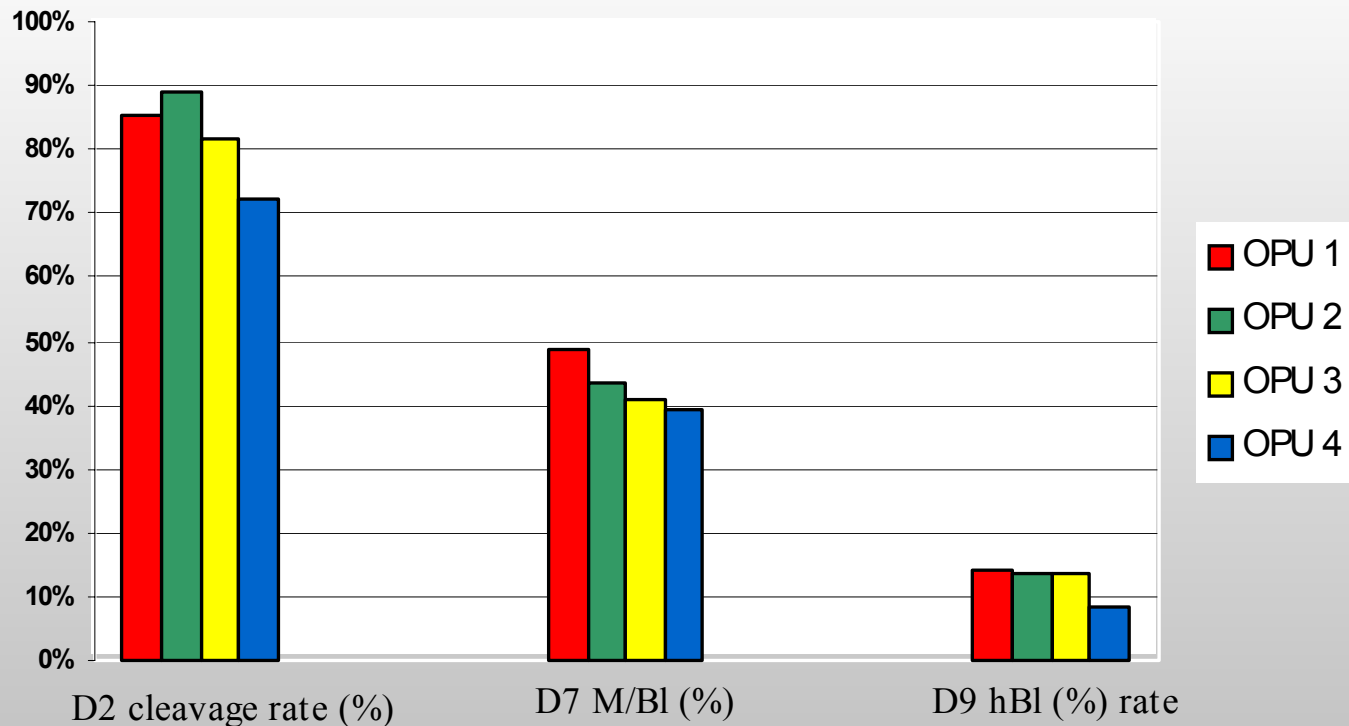
CONCLUSION

The repeated stimulation prior to OPU does not affect the follicular dynamic, the yields, morphological quality and developmental competence of COCs after reapeted superovulation with FSH-P in Simmental heifers.